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STUDIES ON DIPTEROUS PARASITES OF THE SPRUCE BUDWORM, CHORISTONEURA FUMIFERANA (CLEM.) (LEPIDOPTERA: TORTRICIDAE)

V. OMOTOMA FUMIFERANAE (TOT.) (DIPTERA: TACHINIDAE)[1]

H. C. COPPEL AND B. C. SMITH[2]

Abstract

Omotoma fumiferanae (Tot.), a parasite of a small number of Lepidoptera in North America and the most common of the native tachinid parasites of *Choristoneura fumiferana* (Clem.) in British Columbia, deposits macrotype eggs on the integument of the host. The time from oviposition (on a host about to pupate) to puparial formation was 8 to 12 days at 23° C. and a relative humidity of 60%. Approximately 25% of puparia produce adults the same year. The fate of these adults is not known. The remainder of the puparia produce adults the following spring. Among the important characters for identifying the immature stages of *O. fumiferanae* are the buccopharyngeal apparatus and the anterior and posterior spiracles.

Introduction

The tachinid parasite *Omotoma fumiferanae* (Tot.) was listed by Wilkes, Coppel, and Mathers (20) and by Coppel (4) as first in importance in British Columbia among the dipterous parasites of *Choristoneura fumiferana* (Clem.) and second and third respectively among all parasites of this species. It is responsible for a parasitism of up to 7%, or 44% of the total. Dowden, Buchanan, and Carolin (7) and Simmonds and Smith (15) also placed *O. fumiferanae* among the most important dipterous parasites of the budworm in Colorado, U.S.A. The species is present but not abundant in Eastern Canada. The species was reared at the Belleville laboratory from budworm collections made in Western Canada and Colorado, U.S.A., for release in infested areas in Eastern Canada where it was not common. More than 8000 adults have so far been released in New Brunswick, Newfoundland, Ontario, and Quebec, most of them in New Brunswick and Ontario. A small shipment was also sent to New York State, U.S.A.

Specimens from British Columbia reared at the Belleville laboratory on *C. fumiferana*, *Pyrausta nubilalis* (Hbn.), and *Pieris rapae* (L.) provided material for most of the descriptive and biological data given in this paper.

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Systematic Position, Distribution, and Hosts

Tothill (17) described a new tachinid species under the name *Winthemia fumiferanae* in 1912, from a series of 18 males and 18 females reared from the spruce budworm collected in British Columbia and Quebec. Walton (19), in 1913, examined Tothill's cotypes and distinguished *W. fumiferanae* from *Winthemia quadripustulata* (F.). In 1931, Reinhard (12) redescribed *W. fumiferanae* and listed *Nemosturmia pilosa* Tns., described from New Hampshire, as a synonym. Reinhard stated that *W. fumiferanae* was closely related to *Omotoma amoena* (Mg.) of Europe and that these two might even be conspecific, but that it was expedient to consider them distinct. In 1936, Townsend (18) stated that both *N. pilosa* and *W. fumiferanae* belong to the genus *Omotoma* and that *fumiferanae* is structurally inseparable from *O. amoena* and is the "western representative" of *pilosa*.

O. fumiferanae appears to be confined to the temperate areas of North America. It has been recorded as a parasite of the spruce budworm from coast to coast in Canada and the United States (4, 7, 12, 15, 17, 20). It has been listed as a parasite of other lepidopterous hosts in the eastern United States, from New Hampshire, Vermont, Massachusetts, and New Jersey (14), and in Canada, from Manitoba and Saskatchewan (11).

O. fumiferanae has been recorded only from lepidopterous hosts. Schaffner and Griswold (14) and Thompson (16) listed seven North American hosts, all of the families Geometridae, Noctuidae, and Tortricidae. In Canada, *O. fumiferanae* has been reared from *C. fumiferana* and the large aspen tortrix, *Choristoneura conflictana* (Wlkr.) (11).

Description of Stages

Adult

A key to separate the adults of the four species of *Winthemia* known in North America was provided by Tothill (17) in 1912. In 1931, Reinhard (12) revised the genus and provided a key for 32 species, 16 of which were new. At the same time, Reinhard adequately redescribed the adult male and female of *W. fumiferanae*.

Egg

The egg (Fig. 1) is macrotype, 0.4 mm. wide, 0.7 mm. long, and white. In dorsal view it is oval, whereas in lateral view it is flat ventrally and arched dorsally. The ventral surface, which is in contact with the host integument, is thinner and more flexible than the dorsal surface. A hexagonal pattern of reticulations is visible on the dorsal surface at a magnification of 40 ×. A group of approximately 15 cell-like structures with rounded walls (Fig. 2) is present dorsally at the anterior end of the egg. These are similar in position to the micropylar openings recorded in *Phorocera hamata* (A. and W.) by Baldwin and Coppel (3).

TABLE

APPROXIMATE NUMBERS OF ROWS OF SPINES ON THE DORSAL, LATERAL, AND VENTRAL REGIONS OF *O. fumiferanae* LARVAE

Stage	Band	Position	Segment I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	Anterior	Dorsal	6–8	3+2	1+4	1+4–5*	3+5	4+5	5+3	2+6	2–3+2	2+3	2–3
		Lateral	6–8	4–5+3	2+3	2+4	1+4	4–5	4–5	4–5	5–6	4–5	0
		Ventral	6–8	5–6	2+4	3+3	3+2	2+3	5+3	5	5	5	8
	Posterior	Dorsal	0	0	0	0	1–2	0	0	0	0	0	2
		Lateral	2–3	0	0	0	2	2–3	2	2+3	1+3	3	0
		Ventral	0	0	0	0	0	1	0	0	0	0	0
II	Anterior	Dorsal	6	2+4	4	2	2	1+1	1+1	1–2	1–2	3–4	3–4
		Lateral	0	0	0	0	0	0	0	0	0	0	0
		Ventral	2+6	2+5	6+4	5–6	3+2	3	5–6	3–4	2–3	2+3	3–4
	Posterior	Dorsal	0	0	0	0	0	0	0	0	0	2–3	6–8
		Lateral	0	0	0	0	0	0	0	0	0	0	5–6
		Ventral	0	0	0	0	2	2–3	3–4	4–5	2–3	2–4	7–9
III	Anterior	Dorsal	4+1	3–4	4+1	4+1	2+2	2+1	0	0	0	0	0
		Lateral	4–5	2–3	5	3+2	2–3+1	2–3	1–2	0	0	.0	0
		Ventral	6+3	6+1	4–5+1	4+1–2	3+2	3+3	2–3+2	1–2	1–2	0	0
	Posterior	Dorsal	0	0	0	0	0	1–2	1–2	1–2	1–2	7–8	10
		Lateral	0	0	0	1–2	2–3	2–3	1–2	2–3	2–3	7+2	8
		Ventral	0	0	0	0	1–2	1–2	1–2	1–2	2–3	7–8	12

*One continuous row plus four or five discontinuous rows.

First Instar

The body of the larva tapers anteriorly and is somewhat truncate posteriorly, resembling in shape that of *Madremyia saundersii* (Will.) (6). It varies in length from 0.63 mm., immediately after eclosion, to 2.17 mm., when fully fed and about to molt. The body is stoutest at the first and second abdominal segments and tapers anteriorly to the short conical pseudocephalon and posteriorly to the bluntly rounded 11th segment. The cuticle is opaque and unpigmented. Each segment bears an armature of black spines in either continuous or discontinuous transverse rows (Table I). These rows of spines are on the anterior margins of all segments and on the posterior margins of some. The anterior spines are directed posteriorly and the posterior ones anteriorly.

The pseudocephalon bears scattered spines and a pair of bulblike sensory organs.

The buccopharyngeal apparatus (Figs. 3 and 4) is deeply pigmented and is unjointed. It extends posteriorly into the first two thoracic segments and varies in length from 0.15 to 0.19 mm. It may be divided into three regions for convenient description. The anterior region is in the form of a single median hook approximately one-third as long as the apparatus. This hook tapers anteriorly from dorsal and ventral notches that mark the junction of the anterior and the intermediate regions. Its dorsal edge is serrated anteriorly to form a sawlike structure with about 12 teeth. The intermediate region consists of two trunklike arms each of which arises from the base of the median hook. A salivary plate is present below the arms. The salivary plate and two spindle-shaped plates one at each side of the hook comprise the deeply pigmented plates associated with the buccopharyngeal apparatus. Two foramina are usually visible on the lateral surfaces at the junction of the anterior and intermediate regions. The posterior region consists of two dorsal and two ventral wings. The dorsal wings are broader and more deeply pigmented than the ventral ones.

The first instar larva is metapneustic. There are two spiracles on the posterior surface of the 11th segment. Each spiracle (Fig. 5) has two simple openings. The felt chambers are lightly pigmented and are visible through the body wall. Each is approximately three times as long as wide.

Second Instar

The second instar larva resembles the first in form. The length averages 3.64 mm. and ranges from 2.88 to 4.40 mm. The cuticle is more opaque than that of the first instar, though the felt chambers and the buccopharyngeal

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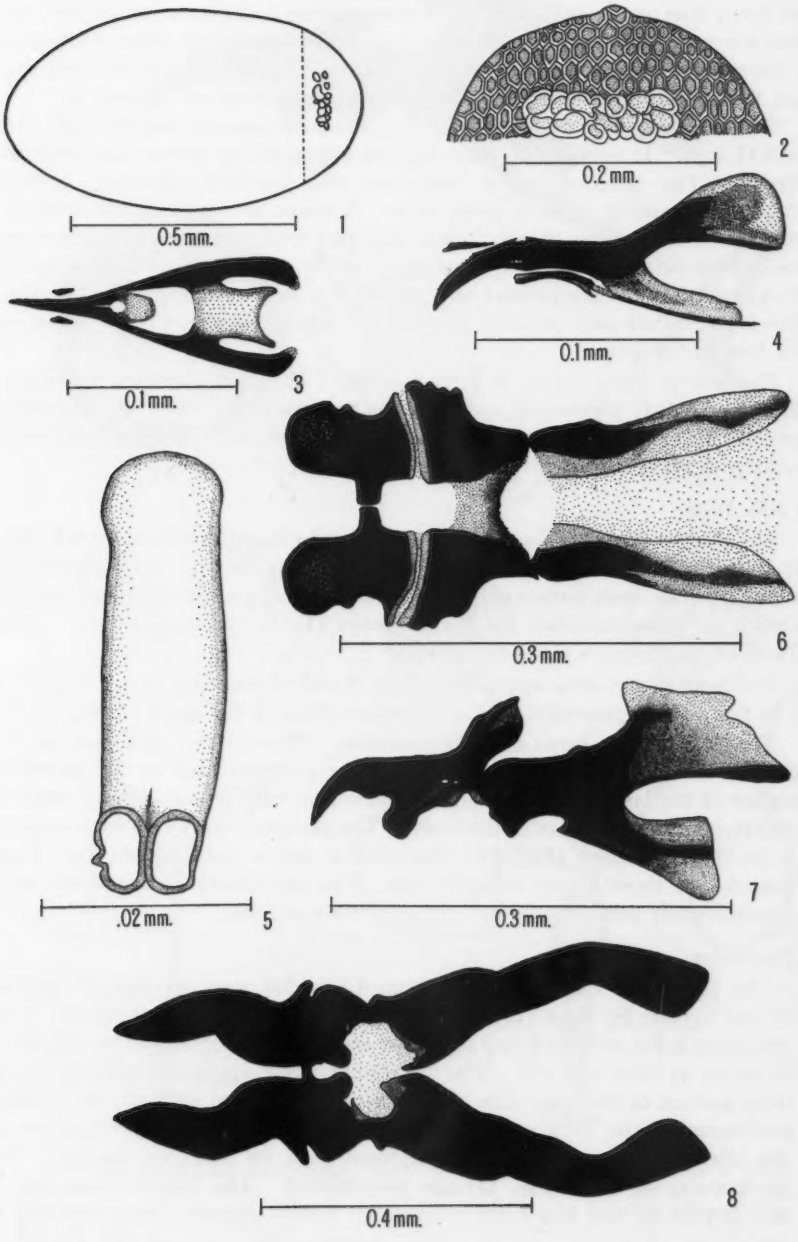
FIGS. 1–8. *Omotoma fumiferanae* (Tot.). 1. Egg, dorsal view. 2. Egg, anterodorsal surface enlarged to show micropylar cells. Semidiagrammatic. 3. Buccopharyngeal apparatus of first instar larva, dorsal view. 4. Buccopharyngeal apparatus of first instar larva, lateral view. 5. Felt chamber of first instar larva showing posterior spiracle. 6. Buccopharyngeal apparatus of second instar larva, dorsal view. 7. Buccopharyngeal apparatus of second instar larva, lateral view. 8. Buccopharyngeal apparatus of third instar larva, dorsal view.


0.5 mm.
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0.1 mm.
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0.3 mm.
7
0.4 mm.
8

apparatus are still visible through the body wall. In proportion to its size the larva does not appear to have as many spines as the first instar, but the rows are more distinct. The number of rows of spines on the anterior margins of the segments decreases progressively toward the posterior end of the body, and those on the posterior margins toward the anterior end (Table I).

The buccopharyngeal apparatus (Figs. 6 and 7) ranges in length from 0.24 to 0.37 mm. It consists of three regions separated by dorsal and ventral notches. The anterior region comprises two ventrally directed, curved hooks, each arising from a stout base. A blunt process extends dorsally from the basal region of each hook and two ventrally directed processes, one on each side, are present approximately midway along the ventral margins. Two oval foramina are present on the lateral surfaces of the anterior region. The intermediate and posterior regions are similar in structure to those of the first instar larva.

The second instar larva is metapneustic. The two posterior spiracular plates are lightly pigmented and each has two openings. The felt chambers are comparatively short, and each is approximately one and one-half times as long as wide.

Third Instar

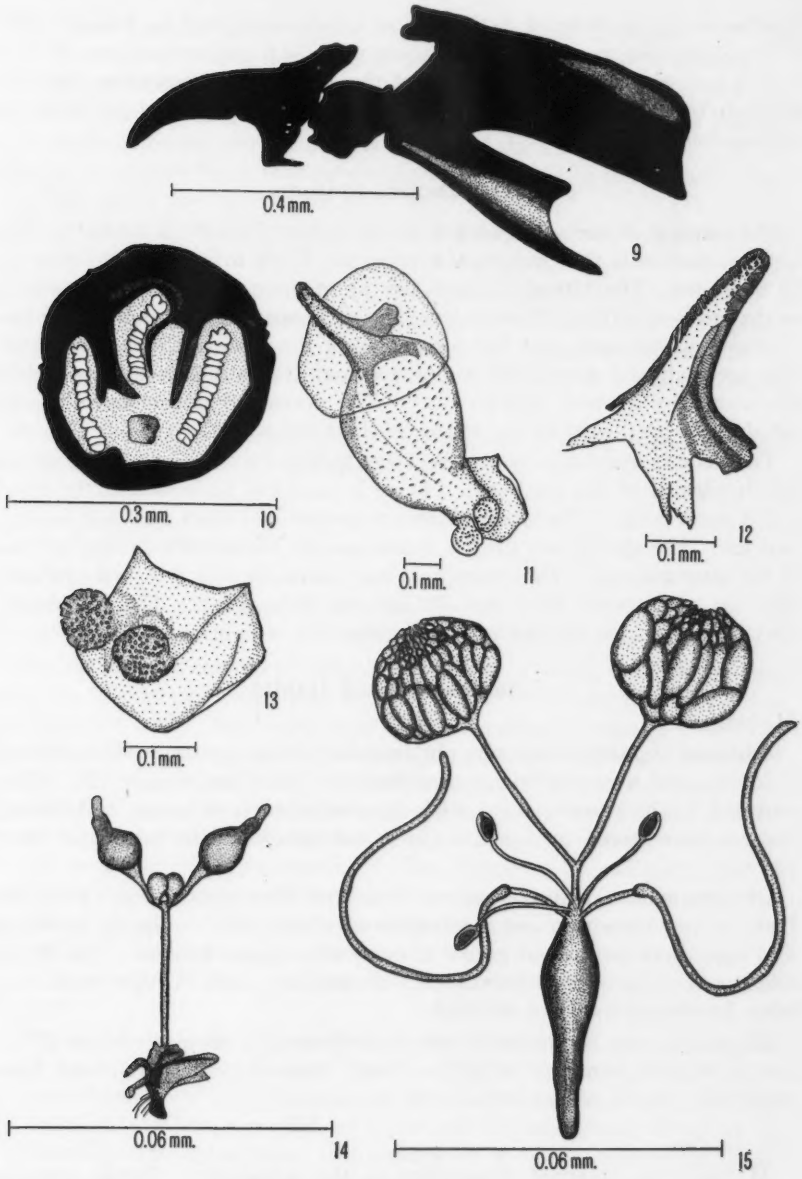
The third instar larva is robust and ranges in length from 8.1 to 9.8 mm. It is darker in color than those of the preceding instars. The abdominal segments have weak lateroventral pseudopodia that appear to be structurally similar to those described for *Bessa harveyi* (Tns.) by Hawboldt (8). The distribution of spines is shown in Table I.

The buccopharyngeal apparatus (Figs. 8 and 9) has an average length of 0.76 mm. It is generally similar in form to that of the second instar larva.

The third instar larva is amphipneustic. The anterior spiracles are on the lateral surfaces of the third segment, the posterior ones on the posterior surface of the last. Each posterior spiracle is circular in outline and projects slightly above the surface of the body. The peripheral region of each spiracle is darkly pigmented (Fig. 10), the central region only lightly so. Each spiracle has three nearly straight slits. The felt chambers are short, each approximately one and one-half times as wide as long.

Puparium

The puparium varies in length from 4.5 to 7.0 mm., averaging 6.4 mm. It was figured by Ross (13). It is subelliptical and chestnut-brown. The pseudocephalon and the larval segments are readily recognizable by the bands of spines at their margins. The buccopharyngeal apparatus adheres to the inner surface of the cap. The anal aperture is situated ventrally on a slight protrusion of the 10th segment. The anterior spiracular openings are on the lateral surfaces of the third segment near its posterior margin. The prothoracic spiracles (Fig. 11) are protuberant. The cornicle (Fig. 12) is well developed and has many respiratory orifices apically along one-half to two-thirds its length. Each internal spiracle (Fig. 13) has two groups of



FIGS. 9–15. *Omotoma fumiferanae* (Tot.). 9. Buccopharyngeal apparatus of third instar larva, lateral view. 10. Spiracular plate of third instar larva. 11. Pupal respiratory apparatus. 12. Tip of prothoracic cornicle. 13. Internal spiracle. 14. Internal reproductive system of male. 15. Internal reproductive system of female.

papillae similar to those of *Actia diffidens* Curran as figured by Prebble (10). Each group arises separately and contains 40 to 70 papillae either in a circular or in a radiating fashion at the end of the atrium. The posterior spiracles protrude from the posterior surface of the last segment dorsad of the longitudinal axis.

Reproductive Systems

The internal reproductive system of the female (Fig. 15) is similar to that of other tachinids that lay macrotype eggs. Each ovary contains at least 16 ovarioles. The lateral oviducts are approximately three times as long as the median oviduct. The ducts of the three spermathecae and of the two accessory glands open into the dorsal wall of the uterus at its anterior end. The spermathecae are darkly pigmented and are oval. Each is joined to the uterus by a short, narrow duct. The accessory glands are elongate-tubular and are joined to the uterus by short ducts.

The male reproductive system is shown in Fig. 14. The paired testes are lightly pigmented and are ovoid. There is a tail-like projection at the distal end of each testis. The lateral ducts are narrow and short and lack seminal vesicles. The ejaculatory duct is approximately three times as long as each of the lateral ducts. The accessory glands are saclike and their ducts open into the ejaculatory duct near its anterior bifurcation. The ejaculatory duct opens into the ejaculatory pump, which is anterior to the penis.

Life History and Habits

Methods

Adults of *O. fumiferanae* were obtained for life history studies from British Columbia and were handled as described by Arthur and Coppel (2). They were fed crushed raisins and a 10% aqueous solution of honey. Males and females were stored in separate cages and sprayed with tap water twice daily.

Observations on mating were made as with *Phryxe pecosensis* (Tns.) (9). Data on preoviposition and oviposition were obtained by placing budworm and other host larvae and pupae in cages with mated females. The larvae and pupae of *Galleria mellonella* (L.), *P. nubilalis*, and *P. rapae* were used when budworms were not available.

All rearing was conducted in the laboratory at a temperature of 23° C. and a relative humidity of 60%. Daily dissections of parasitized hosts provided a record of development of the parasites.

Life History

Mating was observed frequently in the laboratory. Newly emerged females mated readily with males 2 days or more old. As the unmated females aged, matings became increasingly infrequent. Only one instance was noted of a female mating more than once. Mated pairs remained in copula for 15 to 45 minutes.

The preoviposition period varied from 8 to 11 days, averaging 10. The oviposition period ranged from 2 to 19 days, averaging 10 for 14 females.

At oviposition, the embryo is relatively undeveloped, but during the next 48 to 72 hours development is rapid. Though some of the eggs hatched in 72 hours, hatching usually occurred within 5 days of deposition. In one instance, a parasite larva that subsequently developed to the puparial stage remained within the chorion for 36 days.

The total time from oviposition to the beginning of the formation of the puparium was 8 to 12 days, the average being 10.25. The duration of the first instar was 1 to 2 days; of the second, 2 to 4; and of the third, 4 to 7.

The puparium was fully formed and pigmented within 12 hours. Approximately 72 to 75% of the puparia remained in diapause over winter; the remainder produced adults in 20 to 25 days. The ratio of females to males was fairly constant at 54:46.

The life span of ovipositing females varied from 5 to 39 days, averaging 22.4 for 24 females; males lived up to 25 days.

Habits

Laboratory Observations

Mating, which is not necessarily accompanied by a courtship period, is similar to that of *Ceromasia auricaudata* Tns. as described by Coppel and Maw (5). Individual males may fertilize several females.

After the preoviposition period, the female lays small numbers of eggs each day. Up to eight eggs were laid in 1 day by a female but the average daily rate was 2.03. The larger daily rates were those of females with short life spans. Many mated females laid no eggs, possibly because of inadequate food as Allen (1) recorded for *W. quadripustulata*. The average number of eggs deposited by a female was 25.2 and the range four to 34 for 14 females observed. Eggs were deposited on larvae of all the hosts previously mentioned but not on pupae of *G. mellonella*. When larvae of *G. mellonella* and *C. fumiferana* were exposed simultaneously to females of *O. fumiferanae*, the more active larvae of *G. mellonella* received the larger number of eggs. The preferred site of oviposition is in the thoracic region and up to 15 eggs may be deposited on a single host. An adhesive substance fixes the egg to the host integument.

The young larva does not usually leave the chorion until the host begins to pupate. The parasite emerges from the ventral surface of the chorion and immediately enters its host, usually below the anterior end of the egg. First instar larvae had some difficulty piercing the integument of larvae of *G. mellonella*. The other hosts, particularly *P. nubilalis*, were more satisfactory. A breathing funnel continuous with the entrance puncture ensheaths the larva until it is part way through the third instar, when it breaks free from the funnel and feeds on the vital organs until full-grown. The larva then leaves the host larva or pupa through a rupture in the integument and searches for a pupation site. Pupation, in nature, takes place in the soil.

Sawdust was used in the laboratory. The formation of the puparium does not differ markedly from that reported by Maw and Coppel (9) for *P. pecosensis*. On emergence, the adults are very lightly pigmented except for the spines and bristles. Pigmentation is complete within 6 to 12 hours.

Field Observations

Adults of *O. fumiferanae* from overwintered puparia appear in the field in British Columbia late in May and throughout June, when the budworm larvae are maturing. At elevations of over 4000 ft. the parasites are present well into July. Though most of the progeny from these adults overwinter in puparia in the ground, a few emerge as adults in mid-August. The fate of these or of any progeny they might produce is unknown. Resting adults of *O. fumiferanae* may be captured readily on cool days on surfaces exposed to the sun, such as roadbeds, fence posts, and tree trunks. Their flight is characterized by an audible hum.

Preliminary investigations indicated that superparasitism was very common. Many budworm larvae collected in the field had 12 or more eggs of *O. fumiferanae*. Not more than two larvae completed their development in any one host, and the usual number was one. Multiparasitism was also observed in that budworm larvae parasitized by both *O. fumiferanae* and the external parasite *Phytodietus fumiferanae* Roh. were collected. In very few of these instances did both parasites complete development. Rearing of the remainder indicated that the parasite farthest along in development matured first and left insufficient food for the other. Approximately 1% of the budworm larvae visibly parasitized by *P. fumiferanae* produced puparia of *O. fumiferanae*. It is not known to what degree, if any, *O. fumiferanae* distinguishes between parasitized and non-parasitized budworms. Hyperparasitism of *O. fumiferanae* was observed only in 1947, when *Nasonia vitripennis* (Wlk.) (Hymenoptera: Pteromalidae) emerged from many of the puparia of *O. fumiferanae* collected in British Columbia.

Incidence of Parasitism

Data were obtained on the distribution and abundance of *O. fumiferanae* in infestations of the budworm in Canada. The parasite was reared from budworm collections made in British Columbia, Ontario, Quebec, and Newfoundland, and adults were captured in the budworm-infested areas of New Brunswick. It has also been reared from collections made in Colorado, U.S.A. (15).

The bulk of the data on abundance was obtained from large-scale collections of the budworm made annually in British Columbia from 1944 to 1949. Parasitism by *O. fumiferanae* varied with localities and years in British Columbia. However, this species was consistently at or near the top of the list of parasites in abundance. In mass collections made at Mt. McLean the percentage parasitism by *O. fumiferanae* was: 1944, 0.89; 1945, 6.48; 1946, 3.43; 1947, 3.38; 1948, 2.67; 1949, 4.73. This represents up to 30% of the parasitism by all species. At Texas Creek the percentage parasitism

was 1.70 in 1947, 5.68 in 1948, and 7.32 in 1949, representing 8.80, 37.07, and 44.14% of the total parasitism during these years. The same general situation existed at Fountain Valley from 1947 to 1949, when parasitism by *O. fumiferanae* was 1.63, 3.63, and 2.69% in the 3 years. Mass collections from Mission Mountain in 1945 and McGillivray Falls in 1944 showed percentage parasitism of 13.55 and 14.86% respectively. Collections of budworms at 7-day intervals from most of the above areas showed parasitism by *O. fumiferanae* of much the same magnitude as that for the mass collections. No significant differences in parasitism by *O. fumiferanae* were found at various altitudes within the Douglas fir and alpine fir–Engelmann spruce zones, where the budworm population was the highest. Percentage parasitism was 3.22 at 1000 ft., 2.04 at 2000, 3.45 at 3000, and 3.31 at 4000. A preliminary investigation showed that *O. fumiferanae* was responsible for 4.59% parasitism of budworms collected from Engelmann spruce, 2.88% from Douglas fir, 1.73% from alpine fir, and 0.0% from juniper.

In Ontario, collections of budworms made near Belleville showed that percentage parasitism was 0.30 in 1947, 2.13 in 1948, and 0.80 in 1949. Total parasitism was very low in each of the years, and in 1948 *O. fumiferanae* contributed 20% of the total.

In Quebec, collections of budworms made at Maniwaki showed that percentage parasitism was 1.30 in 1947, 1.50 in 1948, 3.41 in 1949, and 7.0 in 1950. By 1950 this species provided one-quarter of the total parasitism. A similar situation was recorded for collections made at Forbes Depot in 1948 and 1949. Parasitism at Mobert in 1947 was 8.53%, and at St. Germaine in 1950 it was 3%.

Collections of budworms received at Belleville from Bowring park, Newfoundland, showed that percentage parasitism was 0.10 in 1947, 0.49 in 1948, and 0.84 in 1949. Only in 1948, when the total parasitism was low, did this species appear to become more important, contributing approximately 15% of the total.

O. fumiferanae is probably present in all budworm populations in Canada. Its numbers fluctuate widely, from rare to common in Eastern Canada, to abundant or often the most important parasite in Western Canada.

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RESPIRATORY MOVEMENTS AND ENDURANCE OF FROGS AT LOW PRESSURES[1]

JAMES C. HALL AND HELEN I. BATTLE[2]

Abstract

Exposure of three species of frogs (*Rana pipiens*, *R. clamitans*, and *R. sylvatica*) to pressures of 1.5, 3.0, and 5.0 cm. Hg induces characteristic changes in the frequency and type of breathing. The immediate initial effect is that of increasing both mouth and lung oscillations for a brief period, prior to a gradual decline in rate. Lung oscillations persist longer than mouth oscillations, and on return to normal atmospheric pressure, are initiated earlier and at a rapid rate. During advanced stages of decompression, excess lung inflation is resultant from several consecutive inhalations, prior to a deep exhalation. *R. pipiens* is most resistant to low pressure, and *R. sylvatica* least resistant. Large individuals of a species survive longer than small. Survival is better with little oxygen in an atmosphere of nitrogen at normal atmospheric pressure than with a comparable oxygen supply at a reduced pressure. A brief acclimatization to pressures of 1.5 and 3.0 cm. Hg can be produced by prolonged exposure to 15 cm. Hg but not by intermittent exposures to low pressure.

Introduction

Few investigations have been carried out to determine the effects of low pressures on Amphibia. Gordon (8) observed a diminished activity in *Necturus* maintained at a pressure of 33.0 cm. of mercury over a period of 9 weeks. Some acclimatization was evident after 4 or 5 weeks and an increase in the numbers of red cells and of red cell progenitors reached a maximum in 7 weeks. Vellard (15) described some anatomical variations in amphibia living at high altitudes in the Andes. Cole and Allison (6) found no variation in the respiratory rate of frogs during small fluctuations in barometric pressure.

More work has been done on the closely related problem of the effects of oxygen lack. Pflüger (11), Sokolows and Luchsinger (14), and Aubert (1) described the actions of frogs in atmospheres low in oxygen but did not refer to an acceleration of respiration previously noted by Rosenthal (12). Babák (2, 3) found that the respiratory movements of frogs increased during oxygen lack, and he also recorded in detail the different phases in respiration during exposure to an atmosphere of pure hydrogen. Since Winterstein (16) maintained that carbon dioxide produced no effect on the respiratory center, Babák (2) concluded that oxygen lack was the dominant factor in respiratory control. Smyth (13), repeating many of Babák's experiments, demonstrated that the effects of oxygen lack could be at least partly ascribed to reflexes originating in the carotid gland. Krogh (10) had calculated that approximately two-thirds of the carbon dioxide is excreted through the skin.

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Accordingly, Smyth (13) concluded that the oxygen tension in the blood is the main factor influencing the respiratory center, since, because of the peculiar vascular arrangements in the frog (7, 9), high concentrations of carbon dioxide never come in contact with it. Bastert (4) determined that diving in frogs is made possible by an automatic regulation of the size of the capillaries, dilation being caused by oxygen lack, contraction by oxygen excess. The long period of apnea during diving does not result in an oxygen deficit, since the amount of oxygen taken up by the blood was found to be equal at all oxygen percentages between 18 and 8%.

There is ample evidence, therefore, that frogs possess a great ability to exist with extremely small quantities of oxygen. The experiments reported here were designed to determine the resistance of these amphibians to extreme decompression and coincidentally to compare the respiratory movements at low pressures with those during oxygen lack at normal atmospheric pressure.

Materials and Methods

Three species of frogs, namely *Rana pipiens*, *Rana clamitans*, and *Rana sylvatica*, were used in these investigations. These animals were collected south of Georgian Bay in southwestern Ontario. Two sizes of *R. pipiens* and *R. clamitans* were utilized, namely "large", ranging from 40 to 50 g. in weight, and "small", ranging from 3 to 6 g. The *R. sylvatica* specimens corresponded in weight to the "small" group of the other two species.

The effects of decompression were observed with the frog at rest on a glass plate in a vacuum desiccator in which some water was present. The desiccator was evacuated fairly rapidly so that the pressure was reduced to 3.0 cm. of mercury at the end of 4 minutes. The lowest pressure to which the frogs were subjected, namely 1.5 cm. of mercury, took longer to reach because it is approximately the vapor pressure of water at 20° C., the temperature at which the experiments were conducted. The mouth and lung movements of the animals were timed during the course of the experiment for 1-minute intervals with a stop watch. Since Cole and Allison (6) had observed that many external factors affect breathing movements, the frogs were allowed to remain in the desiccator for 15 minutes before decompression was begun, to minimize any effects of handling, etc.

In the first series of tests the endurance time of the animals was determined at pressures of 1.5, 3.0, and 5.0 cm. of mercury, the desiccator being sealed off when the desired pressure was reached; and also at a pressure of 5.0 cm. of mercury with a changing air supply. In the latter, as soon as 5.0 cm. pressure was reached, a small stream of air was admitted to just balance the effects of evacuation. The endurance time was regarded as the period of subjection prior to the cessation of all respiratory movements and loss of posture. Air was subsequently admitted until normal atmospheric pressure was restored and the recovery phases of the animals were noted.

In a second series of tests the desiccator was evacuated in the same manner but at the end of 10 minutes the pressure was restored to 76.0 cm. of mercury

by the admission of nitrogen. Water has a vapor pressure of 17.54 mm. of mercury at 20° C. Accordingly the partial pressures of oxygen at the three reduced pressures employed (i.e. 1.5, 3.0, and 5.0 cm. of mercury) were approximately 0, 0.3 and 0.7 cm. of mercury respectively. The animals in the atmosphere of nitrogen had the same amounts of oxygen available as in the previous series but were maintained at normal atmospheric pressure. Thus the effects of low oxygen partial pressure and of reduced mechanical pressure were separated.

Results

I. Respiratory Movements of Frogs under Reduced Pressure

The respiratory movements of frogs under decompression and recovery from decompression always follow a characteristic course. These changes are described below, using as an example a typical experiment with a large *R. pipiens* subjected to a pressure of 3.0 cm. of mercury (Table I, Figs. 1 and 2).

Decompression Phase

A marked initial increase in the frequency of both mouth and lung oscillations occurs with the initiation of decompression. By the time the final reduced pressure is reached, the frequency of the mouth oscillations has fallen to normal or even below normal. On the whole, however, the lung oscillations show a greater relative increase over a longer period of time. Thus the rate of mouth oscillations returns to normal in 4 minutes while that of the lung oscillations requires approximately 10 minutes. Moreover, the former have only doubled their frequency while the latter increase almost five times and in addition exhibit greater amplitude.

Following the initial rise, the mouth oscillations generally become intermittent, being separated by pauses of 5 to 15 seconds. They gradually decrease in both amplitude and frequency, and finally, prior to complete cessation, are very rapid and shallow. Since the latter rapid phase was observed to occur in only approximately half of the individuals, its significance is questionable. After complete cessation, the only movements of the floor of the mouth are those involved in what is described below as lung "pumping".

Concurrently with the decrease in mouth oscillations a change occurs in the type of lung oscillations. It persists from the time of return to normal frequency after the initial rise to the period when posture is lost and all respiratory movements cease. In normal respiration, inhalation is followed by a rest or quiescent period prior to exhalation. Under decompression, however, the lung movements become grouped with several inspirations occurring in rapid succession without intervening expiratory movements. Moreover, the inspirations are more forceful than normal, so that the lungs are "pumped up" or excessively inflated during a prolonged rest period. Exhalation occurs immediately prior to the next series of inspirations. The inordinately expanded lung, therefore, rests for a longer period of time than normal. During continued exposure to decompression the frequency of the

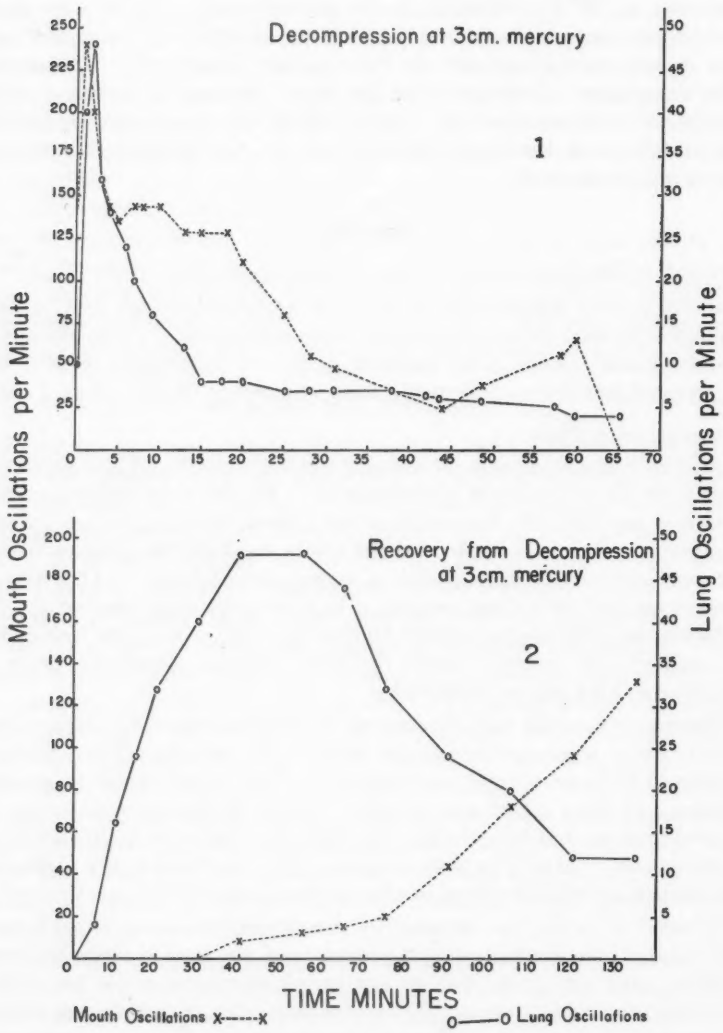

FIG. 1. The frequency of the mouth and lung oscillations of a large *R. pipiens* during the first 70 minutes of decompression at a pressure of 3.0 cm. of mercury.

FIG. 2. The frequency of the mouth and lung oscillations of a large *R. pipiens* during a typical recovery period following loss of posture resulting from exposure for 4 hours to a pressure of 3.0 cm. of mercury.

TABLE I

FREQUENCY OF MOUTH AND LUNG OSCILLATIONS OF A LARGE *R. pipiens* UNDER DECOMPRESSION AT 3.0 CM. HG AND DURING SUBSEQUENT RECOVERY

Time	Mouth oscillations per minute	Lung oscillations per minute
10.00 a.m. (decompression commenced)	140 (normal)	10 (normal)
10.01	240	40
10.02	200	48
10.03	160	32
10.04 (3.0 cm. attained)	144	28
10.05	136	24
10.07	144	20
10.08	144	16
10.10	144	12
10.13	128	8
10.15	128	8
10.18	128	8
10.20	112	7
10.25	80 (pause)	7
10.28	56 (pause)	7
10.31	48 (pauses and shallow)	7
10.38	36 (pauses and shallow)	7
10.44	24 (pauses and shallow)	6
10.48	36 (pauses and shallow)	6
10.58	56 (pauses and shallow)	5
11.00	64 (very shallow)	4
11.05	0	4
3.00 p.m. (posture lost)	None	None
3.05 (atmospheric pressure)	None	4
3.10	None	16
3.15	None	24
3.20	None	32
3.30	None	40
3.40	8	48
3.55	12	48
4.05 (posture regained)	15	44
4.15	20	32
4.30	44	24
4.45	72	20
5.00	96	12 (normal)
5.15	132 (normal)	12 (normal)

grouped inhalations gradually decreases, as well as the number of oscillations per group. In the experiment recorded in Table I the frequency dropped from a group of four per 30 seconds to a group of two once every 3 or 4 minutes at the end of 4 hours. At this time, posture is lost and all breathing movements cease.

Recovery Phase

Air was readmitted rapidly immediately following the cessation of breathing and loss of posture. The rate of lung movements increases enormously before any independent mouth oscillations are evident. At first these have a tendency to be grouped, but later are more evenly spaced. Each movement of the floor of the mouth forces air directly into the lungs, exactly as in "pumping" except that the lungs are deflated between each inspiration. Apparently when the oxygen hunger is partially satisfied, independent mouth oscillations begin, at first singly, and then in groups of two or three between the lung oscillations. As the rate of the latter decreases, the mouth oscillations gradually become more frequent until normal breathing is resumed.

II. Endurance of Different Species of Frogs at Various Pressures

The endurance times of small and large *R. pipiens*, small and large *R. clamitans*, and of *R. sylvatica* were determined at pressures of 1.5 cm., 3.0

TABLE II

ENDURANCE TIME IN MINUTES OF THREE SPECIES OF FROGS AT VARIOUS PRESSURES (CM. HG)

Species	Average initial mouth oscillations per minute	Endurance time in minutes at various pressures (cm. Hg)			
		1.5 cm.	3.0 cm.	5.0 cm.	5.0 cm. with constant air supply
R. pipiens (large)	102±1.5	60±2.6 (9)*	240±4.2 (8)	420±4.2 (8)	480 (9)
R. pipiens (small)	125±3.0	37±1.3 (6)	43±1.3 (7)	60±2.6 (6)	245±4.1 (7)
R. clamitans (large)	61±2.7	46±2.0 (7)	101±4.4 (7)	178±3.4 (7)	324±5.2 (8)
R. clamitans (small)	92±1.4	28±1.8 (7)	45±1.2 (7)	72±1.8 (7)	210±3.5 (7)
R. sylvatica	142±3.7	12±0.6 (9)	18±1.1 (14)	27±1.2 (11)	50±2.1 (10)
R. pipiens (large)		N_2† 82±3.5 (13)	N_2 318±9.6 (10)	N_2 591±15 (10)	
R. pipiens (large) (after 7 days at 15 cm. Hg)		126±2.8 (6)	460±14 (3)		
R. pipiens (large) (after 7 days at 15 cm. Hg; 1 day at 76 cm. Hg)		64±3.5 (3)	268±11 (3)		

*Figures in parentheses below ± sign indicate number of experiments.
†Nitrogen introduced to return pressure to atmospheric after evacuation.

cm., and 5.0 cm. of mercury and at a pressure of 5.0 cm. of mercury with a constantly changing air supply. These data are summarized in Table II. In all instances the pattern of respiratory changes was essentially as described above, i.e. the frequencies of the lung and mouth movements show a rapid initial increase followed by a prolonged decrease, the mouth movements becoming more frequent and ceasing sooner than those of the lungs.

There is a wide variation in the initial rate of breathing movements both with regard to species and size. Small individuals of a species breathe more rapidly than do large ones, but small specimens of *R. clamitans* do not breathe as rapidly as large *R. pipiens*. *R. sylvatica* breathes most rapidly of all the groups tested. This can be correlated with the general activity of the species, *R. sylvatica* being the most active and *R. clamitans* the most sluggish.

There is a direct relationship between the endurance of all individuals and the amount of oxygen available. The respiration and posture of animals having a constant air supply at 5.0 cm. of mercury pressure is maintained for the longest periods. The endurance of large specimens of *R. pipiens* and *R. clamitans* is conspicuously better than that of small individuals of the same species for any one reduced pressure.

There is a very real difference in the endurance times of the different species; both large and small *R. pipiens* are more resistant to decompression than individuals of *R. clamitans* of comparable size. *R. sylvatica* is considerably

more sensitive to reduced pressure than are the other two species. *R. pipiens* exhibits a marked degree of resistance to low pressures, being able to withstand a pressure of 5.0 cm. of mercury when coupled with a small constant amount of fresh air for periods of more than 8 hours. No definite end points were reached in these latter experiments since observations were not continued for longer than 8 hours.

III. Endurance of Frogs at Normal Atmospheric Pressure and Reduced Partial Pressure of Oxygen

A comparable series of experiments was performed with large *R. pipiens* using nitrogen to return the pressure to atmospheric after the desired degree of evacuation was attained (Table II). The endurance times were in all instances longer than for those maintained under reduced pressures, i.e. 82 ± 3.5 to 60 ± 2.6 at 1.5 cm., 318 ± 9.6 to 240 ± 4.2 at 3.0 cm., and 591 ± 15 to 420 ± 4.2 at 5.0 cm. of mercury respectively. Thus the return to normal pressure produced a consistent increase in the endurance time. It is apparent, however, that endurance is dictated much more by the amount of oxygen available than by the over-all pressure.

IV. Acclimatization

The question of acclimatization of individuals both to short-term exposures at low pressures and to long-term exposures at less severe pressures was also investigated. Records were kept of individuals of each of the three different species exposed to pressures of 1.5, 3.0, and 5.0 cm. of mercury during repeated tests, with intervals of approximately 2 days between tests. The endurance times were all essentially the same, varying only a few minutes in either direction. The net change was found to be only 1.5 ± 1.1 minutes. Thus intermittent exposure to extremely low pressures does not appear to result in any acclimatization.

Another group of large *R. pipiens* was subjected for 1 week to a pressure of 15.0 cm. of mercury (i.e. O_2 partial pressure of 2.7 cm. of mercury). During this period the air in the desiccator was changed twice every 24 hours. The endurance times at 1.5 and 3.0 cm. pressure were subsequently determined. The animals were then allowed to remain at normal atmospheric pressure for a day prior to retesting. Comparison of the values given in Table II indicates that the endurance of these specimens was almost doubled by 7 days' exposure to a pressure of 15.0 cm. mercury. This acclimatization was completely or largely lost, however, by exposure for as short a period as 1 day to normal atmospheric pressure.

Discussion

Some of the phases of the respiratory response of frogs to low atmospheric pressure described here are similar to those briefly recorded by Babák (2) and by Smyth (13) using frogs in atmospheres of hydrogen and of nitrogen respectively. Babák referred to the increased frequency and later grouping of lung movements but apparently did not observe the initial increase in

mouth oscillations. Smyth, who recorded lung movements only, obtained similar results. Both investigators noted the marked increase in both the frequency and the amplitude of the lung oscillations immediately after air was readmitted to the chamber.

The change in respiratory movements induced by reduced pressures are probably advantageous to the animal in its struggle for oxygen. The excess inflation of the lungs allows more gas to come into close proximity with the blood, and the delay in exhalation probably ensures that available oxygen is extracted. Bastert (4) showed that the air normally admitted to the lungs in a frog is mixed air, consisting of the "pure" air in the mouth and the air previously expelled from the lungs. When mouth oscillations are absent the air is expelled directly through the mouth to the exterior, and "pure" air is inhaled directly at each breath from the outside. Thus the air is greater in volume, is "purer", and is retained for a longer period than if the normal sequence of respiratory movements was continued. Boelaert (5) has reported essentially similar changes in the repiration of Lacertilia during oxygen want.

It is apparent that most of the responses described here are due to the low partial pressure of oxygen and not to the reduced over-all pressure. One of the most striking observations during these experiments was the almost total absence of any other physical response to the low pressures. The frogs appeared normal and showed no evidence of agitation as the pressure was reduced. Several specimens which failed to recover or recovered only partially were dissected but there was never any evidence of gaseous emboli in the blood vessels.

Although the experiments in which nitrogen was introduced indicated that the return to atmospheric pressure does have a beneficial effect, the endurance times of the animals under different conditions emphasizes the fact that the amount of oxygen available was far more important than the reduced pressure per se. The partial pressure of oxygen necessary for indefinite survival is, however, very low, probably about 1 cm. of mercury for *R. pipiens*. During periods of oxygen lack the animals must derive energy from anaerobic processes.

The three species exhibit obvious differences in endurance to low pressures. If it can be assumed that the rate of respiration gives an indication of the rate of oxygen usage, it follows that *R. sylvatica* should be the most sensitive. This argument, however, cannot be applied to *R. clamitans* which, although it respires more slowly than *R. pipiens*, does not withstand reduced pressures as long.

The acclimatization experiments also raise some questions. Exposure to extremely low pressure for a few hours could hardly be expected to produce any acclimatization especially since 2 days elapsed between successive tests. However, in a few cases the time interval between tests was shortened so that the second test was performed as soon as the animal had recovered from the first. The results were practically identical, thus emphasizing the extreme resistance of these species. The acclimatization produced by

exposure of 1 week to a pressure 15.0 cm. of mercury is rather striking. It was induced in a far shorter time than that recorded for *Necturus* by Gordon (8), but the reduction in pressure was more severe. The surprising feature is that this acclimatization vanished almost completely in 1 day. If it had been attributable to an increased number of red cells, as in *Necturus*, the excess cells would hardly have disappeared in so short an interval.

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EFFECTS OF A HIGH TEMPERATURE ON THE FERTILITY OF DAHLBOMINUS FUSCIPENNIS (ZETT.) (HYMENOPTERA: CHALCIDOIDEA)[1]

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Abstract

Females of *Dahlbominus fuscipennis* (Zett.) were more resistant than males to both the lethal and the sterilizing effects of treatment at 43° C. Three major effects were discovered in the females: permanent sterilization, reduced fertility, and inactivation of the sperm stored in the spermatheca. In addition, temporary sterilization was indicated. Where sterilization occurred in the males it was usually permanent. It was shown that an apparently harmless amount of heat can have a significant effect on reproduction.

Introduction

Many workers have conducted experiments to determine the effects of high temperatures on insect longevity but relatively little work appears to have been done on the effects on fertility.

Young and Plough (7) exposed *Drosophila melanogaster* Mg. to a temperature of 31° C. and found that 96% of the males and 50% of the females were sterilized. No durations of exposure were given and the effect on the males was not permanent. Raichoudhury (4) reared males of *Ephestia kühniella* Zell. at four temperatures and discovered that the sperm from those reared at 30° C. was less abundant and was significantly less motile than that from males reared at 20° C.

The present work, which is an extension of the recent work of Baldwin (1) and Baldwin and Riordan (2), was performed to obtain quantitative information on the effects of heat on the fertility of both male and female insects.

Materials and Methods

The chalcid *Dahlbominus fuscipennis* (Zett.) was used in the experiments since much is already known about some of the effects of high temperature on this species (1, 2). Moreover, the ease with which it can be reared in large numbers by standardized procedures and the parthenogenetic characteristic of the female (unmated females produce only male progeny) greatly simplified designing the experiments.

The insects were reared by techniques similar to those of Wilkes (6) and Baldwin (1). These techniques involve individual parasitization of cocoons of the sawfly *Neodiprion lecontei* Fitch. To obtain females for experiment the host cocoons were opened on the 16th day after parasitization and the *D. fuscipennis* pupae were placed in a shallow box, the sides of which were

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of wood. The top and bottom consisted of sliding glass panels, the bottom being covered inside by blotting paper. Internal dimensions of the box were $8\frac{1}{2}$ in. by $5\frac{1}{2}$ in. by $1\frac{1}{2}$ in. Ventilation was provided by two screened holes, 1 in. in diameter, in one end. Additional adult males were introduced into the box to ensure as high a percentage of matings as was possible. The emerging females were collected into 6-in. glass vials every 24 hours and held a further 24 hours before treatment. Males for experiment were produced by parasitizing cocoons with unmated females; these were obtained by opening parasitized cocoons on the 17th or 18th day after parasitization and removing the adult males immediately. All rearing was carried out at a constant temperature of 23° C. and a saturation deficiency of 5 g. per cu. meter.

The temperature selected for heat treatment was 43° C. and the treatment was administered in the water bath previously used by Baldwin (1) and Baldwin and Riordan (2). Saturated air was supplied to the vials containing the insects at 50 cc. per minute.

Females were treated for 50, 66, 87, 114, and 150 minutes and males for 25, 35, 50, 71, and 100 minutes. The ranges were determined by preliminary experiments, the periods in each range being in logarithmic succession. Sufficient insects were treated for each period to give a minimum survival of 20 apparently unharmed insects for subsequent propagation. After treatment, the insects were held for 24 hours at 23° C. The survivors were then counted to determine mortality.

To determine the effect of the treatment on fertility of the females, 20 apparently unaffected survivors for each treatment period were placed individually with selected *N. lecontei* cocoons. A control was established by similarly propagating 20 untreated females from the same emergence group. The experiments, including the controls, were each repeated 10 times. The cocoons were opened after 15 days and data on the subsequent emergence were recorded. Cocoons in which the host larvae had putrefied or had become desiccated in storage were discarded.

To determine the effect of the treatment on the fertility of the males, 20 survivors for each treatment period were placed individually with virgin females. Twenty-four hours were allowed to elapse before the introduction of *N. lecontei* cocoons, since even under normal conditions the females sometimes begin oviposition immediately they have access to the hosts, whether or not they have been mated (Reeks (5)). As in the treatment of the females, a control was established with untreated insects. To confirm that the females placed with the males were previously unmated, a sample of 20 was used as a check in each replicate. Ten replicates were performed. A further test was run to determine whether any effects on the males were permanent. In this test a group of males was subjected to 43° C. for 50 minutes and 50 of the survivors were placed individually with virgin females on the 1st, 2nd, 3rd, 4th, and 5th days after treatment. Twenty-four hours after the contact between the males and the females, *N. lecontei* cocoons were

introduced. Fifty untreated males were similarly handled to serve as a control. The numbers of cocoons from which only males emerged were recorded.

Males were considered to have been sterilized when the females with which they were mated produced only male progeny.

Results and Discussion

Mortality

Fig. 1 shows that the time required to cause 50% mortality was 110 minutes (19/20 confidence limits 127 and 96 minutes) for the females and 74 minutes (19/20 confidence limits 86 and 64 minutes) for the males. The difference was significant according to the method of Litchfield and Wilcoxon (3).

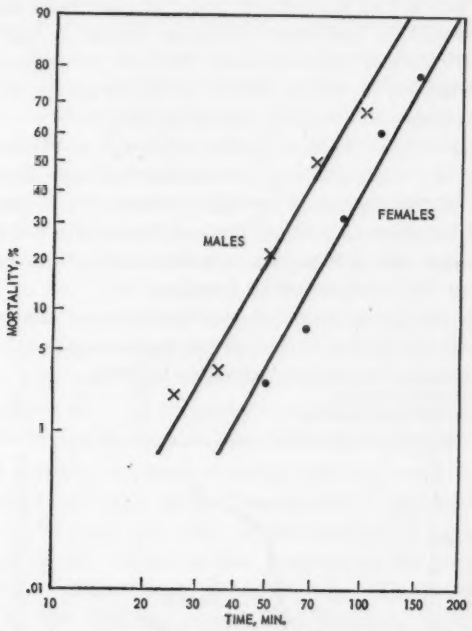

FIG. 1. Time–percentage mortality curves for males and females of *D. fuscipennis* exposed to a temperature of 43° C.

Fertility of Females

The percentages of 200 female survivors for each treatment period showed the following effects:

Duration of treatment, minutes	0	50	66	87	114	150
Percentage sterilized (unparasitized cocoons)	14	25	34	54	70	84
Percentage showing reduced fertility (reduction in progeny)	–	25	38	53	65	82
Percentage with stored sperm inactivated (all-male progeny)	13	13	16	16	38	46

A further effect, temporary sterilization, was suggested by the fact that the peaks of emergence of the progeny for the control insects and for those treated for 50, 66, and 87 minutes fell on the 19th day after the parasites had been placed with the host cocoons, whereas the peaks of emergence of the progeny for those treated for 114 and 150 minutes fell on the 20th and 21st days respectively. This difference was probably caused by temporary damage to those survivors of the longer treatment that did not suffer sterilization or possibly by temporary damage to the eggs themselves resulting in a prolongation of the development time.

Fertility of Males

The only effect on the surviving males was sterilization (Fig. 2), as indicated by the numbers of host cocoons from which only males emerged, proving that the females which had parasitized them had not been mated. Reduced fertility, which would be indicated by higher ratios of males to females in the progeny of survivors of the treatments than in the progeny of the control insects, due to reduction in the quantities of viable sperm, was not revealed, the differences between the ratios not being significant.

The percentages of 200 survivors sterilized in each of the treatment periods are shown in Fig. 2. It is not known whether this sterility was caused by physical damage to the insects or by inactivation of the sperm. The latter is the more likely explanation since the survivors selected for propagation appeared undamaged and in many cases were observed to assume the mating position as soon as they had access to females.

By integrating the percentages of sterilization and mortality caused by the various periods of treatment the percentages of potent males remaining from an initial normal sample are derived as follows:

Duration of treatment, minutes	0	25	35	50	71	100
Percentage potent males remaining	63	62	59	50	8.3	3.1

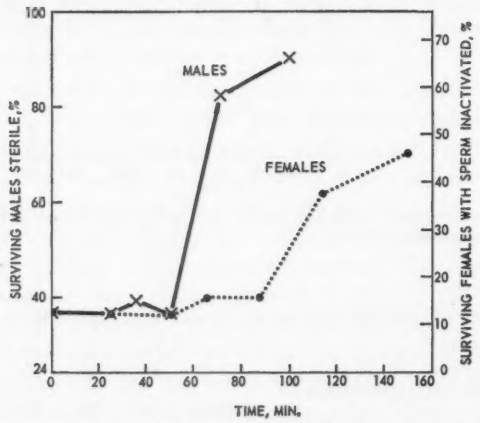

FIG. 2. Percentages of surviving males sterilized and of surviving females in which the sperm was inactivated, by exposure to 43° C. for various periods. The two ordinate scales are placed in relation to each other so as to compensate for the 37% of the males that failed to fertilize females in the controls.

The following data from the test designed to determine the permanency of the effects on the males show that although the major part of the effect was permanent there was in fact a certain amount of temporary damage from which recovery was made (or continued) during 24–72 hours after treatment.

Interval between time of treatment and mating, days	1	2	3	4	5
Percentage of treated males sterile (subsequent all-male emergence)	41	30	41	70	100
Percentage of control males sterile	16	17	29	36	62

Comparison of the Effects on Females and Males

The broken line in Fig. 2 is based on the percentages of treated females in which the sperm held in the spermatheca was presumed to be inactivated. It deviates notably from the line that is based on sterilization of the treated males although this too is presumed to be due to inactivation of the sperm. It is possible that the sperm when stored in the spermatheca is better insulated from high environmental temperatures than when in the vesiscula seminalis. The explanation may, however, not be so simple. It is possible that some other factor, such as greater maturity, is responsible for the apparent increase in the resistance of the sperm. However, the results may also be modified by other unknown factors. For example, the treated females laid progressively smaller quantities of eggs and it may follow that the spermathecae still held a quantity of unused potent sperm. These questions may be resolved by conducting a further series of experiments in which the effects of the treatment are evaluated by dissection of the insects and examination of the sperm in the vesiculae seminalis and the spermathecae respectively. This may also yield information as to the nature of the temporary sterilization noted in the female survivors of the 114- and 150-minute treatments.

Two further important points emerge from the results: (i) At the ED_{50} of the females (110 minutes) it may be calculated that 68% of the survivors are sterilized, whereas at the ED_{50} of the males (74 minutes) 84% of the survivors are sterilized. This further emphasizes the greater susceptibility to damage by heat of the "male element". (ii) When the three permanent effects noted in the females, namely sterilization, reduced fertility, and inactivation of the stored sperm, are integrated with the mortality, which is the only apparent effect, the cumulative or 'real' effect can be derived:

Duration of treatment, minutes	50	66	87	114	150
Percentage mortality	3	7	31	60	77
Percentage reduction in female progeny	38	58	83	97	99.5

The difference between these 'apparent' and 'real' effects is striking and it demonstrates that doses of high temperature which may have comparatively little visible effect on an insect population may in fact significantly influence the size of succeeding populations.

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THE EFFECT OF LOW TEMPERATURE ON THE ASCORBIC ACID CONTENT OF THE OVOTESTIS OF THE SLUG, ARION SUBFUSCUS (DRAPARNAUD)[1]

D. PELLUET

Abstract

The amount of ascorbic acid in the slug *Arion subfuscus* has been estimated quantitatively at laboratory temperatures and at 0°–2° C. The results show that the effect of the exposure to the low temperature reduces the amount present in the ovotestis significantly. In general, the amount of ascorbic acid in the cold treated animals does not exceed that of the controls. This result does not agree with the cytological appearance of the ovotestis exposed to the same conditions, in which the cold treated animals show an increased number of granules of ascorbic acid after an initial decrease.

Introduction

Few studies have been carried out on invertebrate material to compare the cytological demonstration of cytoplasmic materials with a quantitative estimate of their concentration by biochemical methods. The ovotestis of the slug, *Arion subfuscus*, is very suitable material for such a study, since a single ovotestis can be used for sectioning and several of them can be used for biochemical analysis.

Previous work has shown that vitamin C or ascorbic acid is present in the developing eggs of the ovotestis, with very little appearing in either the germinal epithelium or the male germ cells (Pelluet and Watts (3)). Watts (7) showed that slugs which were placed in a cold room at 0°–2° C. contained an increased number of granules of ascorbic acid in the eggs in comparison with the number in the control animals. The granules were counted very carefully and there was an obvious rise in the number, particularly after 10 days at the low temperature. It is of interest to find out the relation between increased numbers of granules demonstrated cytologically and the quantitative changes estimated biochemically, since it is impossible by cytological methods to relate numbers of granules to specific quantities.

Material

Arion subfuscus is an introduced species according to Pilsbry (4) and it has rather a local distribution. According to Hesse, quoted by Pilsbry, all species of this genus are annual, becoming sexually mature in 8 or 9 months, sometimes much earlier. In Nova Scotia *A. subfuscus* has been collected in several localities in Halifax County and a plentiful source of supply occurs at Prospect, a few miles from Halifax. The animals were found in the abandoned gardens of houses which had been removed from the Halifax watershed. Several thousands have been collected from here by lifting up

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Contribution from the Zoological Laboratory, Dalhousie University, Halifax, N.S.

old boards, sheets of tar paper, and debris from the demolished houses, as well as in the long grass of the former gardens.

The slugs were brought back to the laboratory, put into glass dishes with vermiculite and fed on lettuce for 24 hours before being used experimentally; they showed no signs of being affected by their change in environment, since they always ate voraciously and were quite active. After this 24-hour period, the animals were divided into two lots—half in the laboratory and half in the cold room, both groups being given 8 hours of daylight by means of lamps.

Methods

The estimation of ascorbic acid was carried out by the method of Roe and Keuther (6) with a few slight modifications suitable for this material. A known amount of a commercial sample of ascorbic acid was made up into a solution, from which various dilutions were made. These were treated by the Roe and Keuther method, using all the reagents, and the optical density of these known quantities of ascorbic acid were read on a spectrophotometer. From these values a curve was constructed, and this was used to estimate the amounts of ascorbic acid which were found in the ovotestis material.

Samples of material were obtained by cutting off the heads of the slugs with sharp scissors. The motion of the muscles of the body wall quickly forced out the entire viscera and it was easy to remove the ovotestis from its position at the posterior end of the digestive gland without getting any extraneous material.

Ten animals usually supplied enough material for a sample. These samples were done in duplicate as a rule, so that each experiment took at least 40 animals, two samples of 10 animals each from the control and two samples from the animals kept in the cold room. These constituted paired experiments. Samples were also taken from the normal animals to find the amounts of ascorbic acid of these animals kept in the laboratory for varying periods of time and the same was done for animals kept in the cold room. This work was carried out in the summers of 1954 and 1955.

FIG. 1. Amount of ascorbic acid in ovotestes of slugs kept in the laboratory and at 0°–2° C. compared with controls at room temperature. Series A and B were paired, series C unpaired. 1954 data. Number of laboratory days for controls are marked on the bars.

Unhatched rectangles: amount of ascorbic acid in controls.

Hatched rectangles: amount of ascorbic acid in cold treated animals.

Combined unhatched and hatched rectangles: relative amounts of ascorbic acid in controls and treated animals in paired experiments. For example, in B at 14 days the control slug group of the pair had a slightly higher concentration than the specimens in the cold.

FIG. 2. Comparative estimates of amounts of ascorbic acid in the ovotestes of slugs kept at about 2° C. and at laboratory temperatures. 1955 data. For the two bars to the right in F, no controls were available for comparison.

Combined unhatched and hatched rectangles: relative amounts of ascorbic acid in controls and treated animals in paired experiments.

Results

The averages of the duplicate samples are shown on the histograms in Figs. 1 and 2, which include paired and unpaired experiments carried out in 1954 and 1955.

The histograms show that with the exception of Fig. 2D the cold apparently depresses the ability of the animals to synthesize vitamin C.

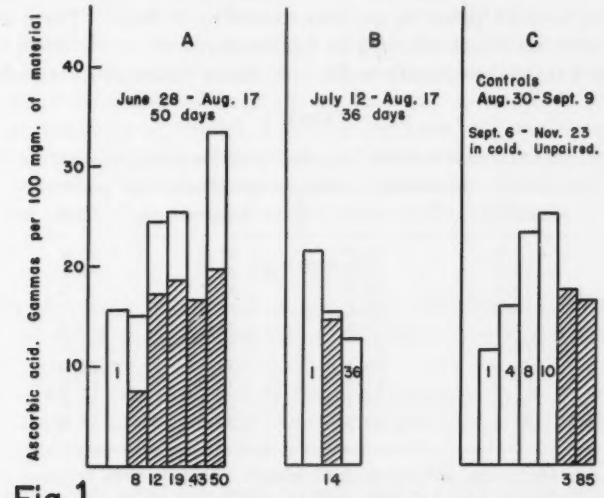

Fig. 1

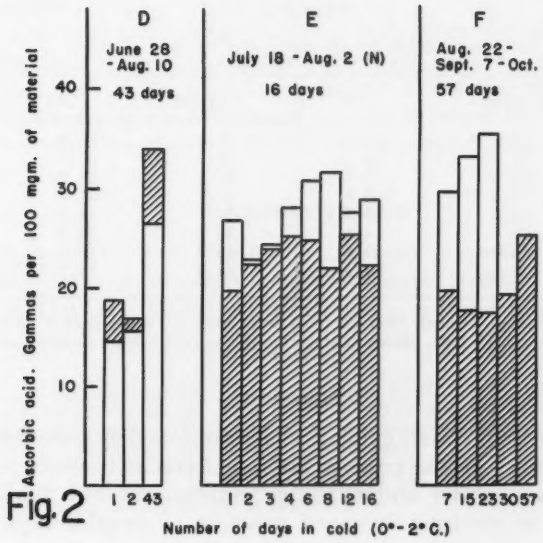

Fig. 2

In order to see whether the cold produced a shock for the first few days, since the animals do not eat or move very actively when first put into the cold room, the figures for the vitamin C level for the first 8 days were treated as one group, and those exposed to the cold for more than 8 days were considered as another group and the t test was applied to each group (Tables I and II). There were 20 pairs of analyses for the vitamin C content of animals exposed to 0°–2° C. compared to the animals kept in the laboratory for 8 days or less, and 16 pairs for periods exceeding 8 days. There is so little difference between these two sets of figures that one is unable to state that for the first 8 days the animals suffer cold shock followed by a recovery.

TABLE I

EFFECT OF COLD ON ASCORBIC ACID IN SLUG GONADS

Control	Cold	Control	Cold
15.1	6.9	23.8	23.7
15.3	9.0	24.5	24.7
12.9	23.3	27.7	25.5
16.8	14.6	28.4	25.3
11.7	16.5	28.5	25.3
19.6	17.1	33.1	24.8
24.3	20.5	30.1	22.1
28.5	19.4	32.9	22.0
23.0	23.1	29.4	20.6
21.8	21.5	30.0	18.8

NOTE: Values are in mg. per 100 g. fresh gonad. Animals were arranged in pairs and exposure to cold was for 8 days or less. Control values were higher than cold and the t test gave a t value of 3.0, showing (for $n=20$) that the effect of cold is to decrease the ascorbic acid significantly.

TABLE II

EFFECT OF COLD ON ASCORBIC ACID IN SLUG GONADS

Control	Cold	Control	Cold
22.5	12.1	27.1	27.6
25.9	22.3	28.4	23.4
24.4	17.7	27.1	22.8
27.3	18.8	22.9	21.8
15.6	15.5	30.4	14.8
15.7	15.1	36.3	20.8
26.4	33.8	36.2	18.0
26.5	34.3	34.9	16.7

NOTE: Same as Table I except that all pairs were kept for more than 8 days. Analysis gives t as 2.8 which, for 16 tests, shows that here also the cold causes a reduction of ascorbic acid.

Discussion

It was hoped that Watts' cytological results would be paralleled by the quantitative results of the present work; unfortunately there is no such agreement. Slugs survive normal winter temperatures, but they appear to do so without synthesizing increased amounts of ascorbic acid. In this

they differ from rats exposed to cold. Dugal and Thérien (1) found that ascorbic acid added to the diet increased the ability of the animals to become acclimatized to the cold, and more recently Monier and Weiss (2) found that rats exposed to 0° C. for experimental periods excreted 53% more ascorbic acid than the controls kept at 21° C.

The discrepancy between the cytological and biochemical results is difficult to explain. However carefully the cytological methods for the demonstration of vitamin C are carried out, there is always a possibility that granules other than the vitamin may be blackened by silver nitrate, or that the cold treatment may have induced physical changes in the cytoplasm resulting in the appearance of artefacts. Reiner (5) carried out a series of experiments on the histochemistry of vitamin C, using test tube and model experiments, as well as animal tissues, and he concluded that while the specificity of the reduction action is reasonably good, the production of diffusion artefacts rendered the test useless for localization on a cytological level.

Summary

1. Estimates of the amounts of ascorbic acid (vitamin C) have been made on the ovotestes of slugs maintained at laboratory temperatures and at 0°–2° C. for varying periods of time.

2. The effect of the exposure to the low temperature is to depress the synthesis of the vitamin, so that there is a significant drop in the amount of the ascorbic acid during the first 8 days.

3. The amount of vitamin C in the ovotestes is generally lower at the beginning of the summer than at the end of the summer, which may reflect the period of maximum egg-laying which takes place in late August and early September.

4. The amount of ascorbic acid in the animals exposed to the cold is significantly lower than in the controls throughout the experimental period.

5. A comparison of the amounts of vitamin C found in the analyses and the cytological picture has been made and the increased number of granules found in the animals exposed to 0°–2° C. is not paralleled by the quantitative results.

Acknowledgments

I wish to thank Mr. J. S. Tait for his assistance in working out the preliminary methods of analysis, when he was a graduate student. I am indebted to Dr. F. R. Hayes for his help in the statistical treatment of the results.

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CHAMBERSIELLIDAE N. FAM. (NEMATODA) WITH EMENDED DIAGNOSIS OF THE GENUS CHAMBERSIELLA COBB, 1920, DESCRIPTION OF C. BAKERI N. SP., AND DISCUSSION OF TAXONOMIC POSITION[1]

K. C. SANWAL[2]

Abstract

Chambersiella bakeri n. sp., collected from the bark of an oak tree in the vicinity of Richmond, Ontario, is described and figured. It differs from *C. rodens* in the structure of the stoma, in the form of the female reproductive system, in the position of the vulva, and in the size of the body. With the new information provided by this species the generic diagnosis of *Chambersiella* Cobb, 1920 is emended. This genus, after emendment, is removed from the family Cephalobidae Chitwood and Chitwood, 1934 and placed in Chambersiellidae n. fam. The relationships of Chambersiellidae with the Rhabditidae and the Cephalobidae are discussed.

Six female and five male nematodes were collected from the bark of an old oak tree growing on the bank of the Jock River in the vicinity of Richmond, Ontario. Examination of mounted specimens showed that they bore some resemblance to *Chambersiella rodens* Cobb, 1920 (4), but that they obviously represented a new species. Further study of this new species revealed that it should be placed in the genus *Chambersiella* but that the description of the genus would have to be emended. This genus is now removed from the family Cephalobidae and is placed in Chambersiellidae n. fam.

Family Chambersiellidae n. fam.

Diagnosis

Rhabditoidea. Lip region with six cephalic cirri, 6 or 10 papillae; anteriormost rhabdions distinct from the rest; stoma broad at the anterior end, gradually narrowing to a vaselike channel posteriorly; posterior part of stoma surrounded by tissue which is differentiated from that of the oesophagus; oesophagus without a distinct swelling at the base of the corpus, i.e., a median oesophageal bulb but with a valvulated terminal bulb; presence of one or two ovaries in the female; amphids opening behind the broad anterior chamber of the stoma.

Type genus: *Chambersiella* Cobb, 1920.

Affinities: The family Chambersiellidae resembles the family Rhabditidae Chitwood and Chitwood, 1937 (2) in the general shape of the oesophagi (which are devoid of a distinct median oesophageal bulb but have a valvulated terminal bulb), in the condition of the female reproductive systems (ovaries being usually paired, rarely single, in the latter family), and in the manner of general distribution of the caudal papillae in the males. It differs, however, from the family Rhabditidae in the possession of cephalic cirri, in the shape

[1]Manuscript received June 4, 1957.
Contribution from Nematology Section, Entomology Laboratory, Science Service, Department of Agriculture, Ottawa, Canada.
[2]Postdoctorate Fellow, National Research Council of Canada.

of the stoma and the arrangement of the stomatorhabdions, in the position of amphidial apertures, and in the absence of caudal alae. It resembles the family Cephalobidae Chitwood and Chitwood, 1934 (1) in the general shape of the oesophagus, in the disposition of the caudal papillae and absence of caudal alae in the male, and in the general appearance of the head region with its cephalic cirri (cephalic probolae of Acrobelinae?), but differs from it in the arrangement of the rhabdions of the stoma, in the position and size of the amphidial apertures, and in the presence of two ovaries in some species.

Genus *Chambersiella* Cobb, 1920, Emended

Diagnosis

Cuticle finely striated with two lateral incisures; lip region with six cephalic cirri and 6–10 cephalic papillae; anteriormost rhabdions of the stoma modified to form hooklike structures and distinct from the rest; posterior part of stoma forming a narrow vaselike glottid apparatus and surrounded by tissue which is differentiated from that of the oesophagus; amphids opening behind the anterior broad chamber of the stoma; oesophagus without a median bulb but with a valvulated terminal bulb; ovaries one or two; vulva slightly pre-equatorial to postequatorial; testis single; male tail with several pairs of caudal papillae; spicules paired and not joined; gubernaculum present; rectal glands present; tail of both sexes with a dorsally hooked terminus.

Type species: *Chambersiella rodens* Cobb, 1920.

Chambersiella bakeri n. sp.*

Females

Length 0.8–1.3 mm.; a = 20–26; b = 3.5–5.6; c = 7.8–10; v = 49.6–53%.

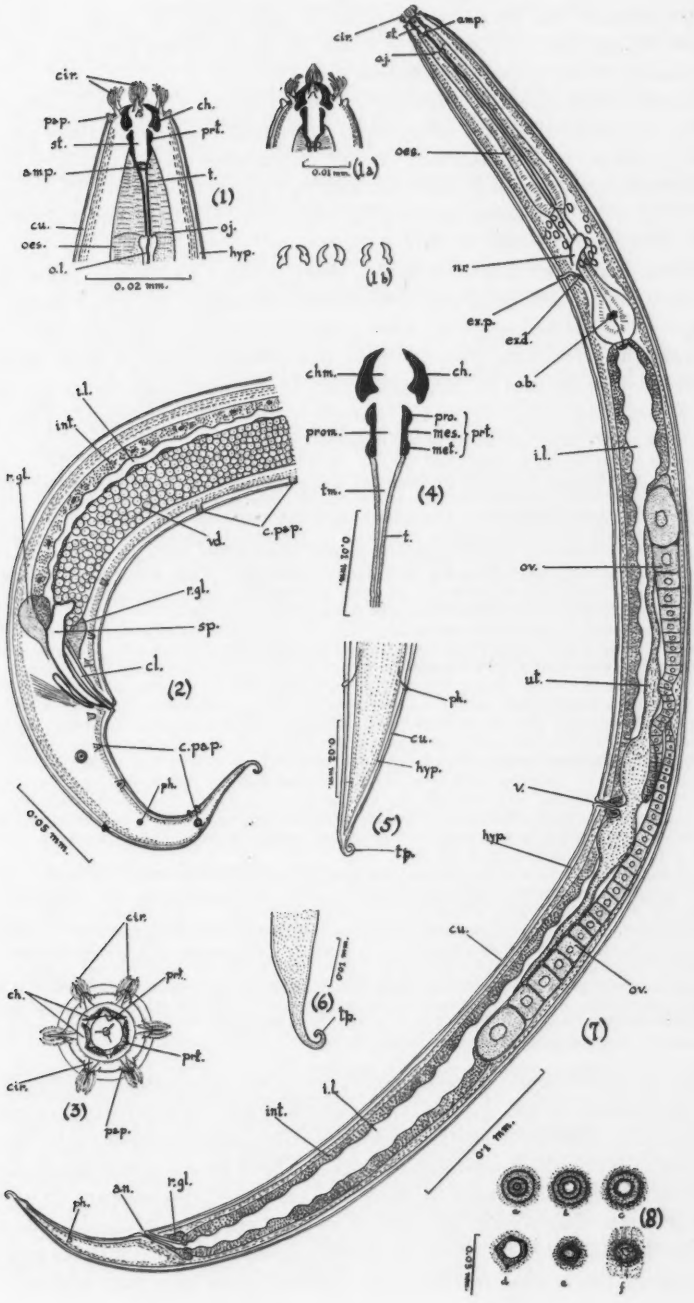
Worms small and translucent. Body gradually attenuated towards both ends, assuming the shape of 'C' when killed by gentle heat. Cuticle very finely striated and bearing two lateral incisures. Cuticular striations 0.0011 mm. apart at the middle of the body.

Head not marked off from the rest of the body and without distinct lips. Cuticle of the lip region with six branched cirri (Figs. 1, 1*a*, 7). Six large

*Named in honor of Dr. A. D. Baker, in charge, Nematology Section, Science Service, Ottawa.

FIGS. 1–8. *Chambersiella bakeri* n. sp. 1. Anterior region of female. 1*a*. Anterior region of female showing slightly projecting hooks. 1*b*. Different views of buccal hooks in different focal planes. 2. Posterior region of male. 3. Head of female, end on view. 4. Stomatorhabdions, lateral view. 5. Tail region of female. 6. Hooked tip of female tail. 7. Female, showing general anatomy. 8. Surface view of vulvar aperture in different focal planes.

ABBREVIATIONS: amp.—amphid; an.—anus; c. pap.—caudal papillae; ch.—cheilorhabdions; chm.—cheilostom; cir.—oral cirri; cl.—cloaca; cu.—cuticle; ex. d.—excretory duct; ex. p.—excretory pore; hyp.—hypodermis; i. l.—intestinal lumen; int.—intestine; mes.—mesorhabdions; met.—metarhabdions; nr.—nerve ring; o. b.—oesophageal bulb; o. l.—oesophageal lumen; oes.—oesophagus; oj.—junction of the oesophagus and stoma; ov.—ovary; pap.—cephalic papillae; ph.—phasmid; pro.—prorhabdions; prom.—protostom; prt.—protorhabdions; r. gl.—rectal glands; sp.—spicules; st.—stoma; t.—telorhabdions; tm.—telostom; tp.—hooked terminus; ut.—uterus; v.—vulva; vd.—vas deferens.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labial papillae on the head, one at base of each cephalic cirrus. (Possibly there are 10 papillae, as figured by Cobb (4) in *C. rodens* but only six were clearly visible in the present specimens.)

Terminal mouth opening leads into a broad stoma (Figs. 1, 7), the walls of which are mostly heavily sclerotized. Stoma divisible into three regions: anterior, middle, and posterior. Walls of anterior region formed by six curved hooks which present different outlines when viewed in different focal planes (Fig. 1*b*). These hooks may lie within the head or they may project slightly (Fig. 1*a*). Walls of middle region of stoma formed by a jointed piece which is distinct from the hooks, there being a visible gap between the two. This part is not uniformly sclerotized. Posterior region of stoma with very lightly sclérotized walls; in some specimens it shows a slight constriction of its walls prior to its junction with the oesophageal lumen (Fig. 4). Whereas the walls of anterior and middle regions of the stoma bound broad chambers, walls of posterior region bound a narrow vase-shaped channel. The three spaces, however, are continuous with each other.

NOTE: My interpretation of the parts of the walls of the stoma (Fig. 4) is as follows: The six curved hooks which constitute the walls of the anteriormost region of the stoma are identified as the cheilorhabdions. These hooks are very probably homologous with the larger and more forwardly extended mouth hooks of *C. rodens*. Cobb (4) called these 'odontia' but used the term 'onchia' for the hooks in *Ungella* Cobb, 1928, indicating that these latter were not labial structures, but rather part of the 'pharynx'. The location and disposition of the hooks in *C. bakeri* in relation to the other sclerotized regions of the stoma leads me to interpret these parts as cheilorhabdions, which have been modified into hooks. The jointed piece which forms the wall of the middle region of the stoma and is separated from the buccal hooks by a visible gap is considered to represent the protorhabdions. In a lateral view the sclerotization of this jointed piece seems to indicate that it is formed by the fusion of three rhabdions. As shown in the figure, the anterior and posterior parts (pro- and meta-rhabdions) show a thicker sclerotization and are consequently more prominent than the middle part (mesorhabdions), which is represented by a narrow bridge between the other two. The lightly sclerotized walls of the posterior region of the stoma are interpreted as the telorhabdions. The light sclerotization of this part may be attributed, as pointed out by Chitwood and Chitwood (3), to the development of pharyngeal musculature, which, in this case, envelops the entire telorhabdions and extends up to the basal part of the protorhabdions. The chambers bounded by the anterior, middle, and posterior regions of the stoma are consequently recognized as cheilostom, protostom, and telostom, respectively. The telostom in this case forms a narrow vaselike glottid apparatus.

The slight constriction on the walls of the posterior part of the stoma in some specimens (Fig. 4) suggests an alternative interpretation. The hooks represent the cheilorhabdions. The heavily sclerotized piece which follows the hooks (prt.) may represent the pro- and meso-rhabdions. The lightly sclerotized walls of the narrow vaselike region (t.), which show a slight constriction in some specimens, may represent the meta- and telo-rhabdions which have fused, the fusion probably taking place at the point of constriction. Both of the interpretations are plausible but no definite conclusion can be reached until more is known about the structure of the stoma of nematodes after a careful study of the developmental anatomy of various groups. The figures in the present paper, however, have been labelled in accordance with the first interpretation.

Stoma opens in lumen of oesophagus, the latter being clearly differentiated from the former (Figs. 1, 7). Oesophagus with a cylindrical corpus and precorpus but without a median bulb. Narrow isthmus swells into terminal oesophageal bulb which contains a valve. Valve shows comb-like striations. Lumen of oesophagus within isthmus and terminal bulb appears to be much narrower than that within the precorpus and corpus. Muscular tissue enveloping the basal part of the stoma differentiated from that of the oesophagus. Oesophageal tissue also seems to be differentiated into two regions. The

tissue of the precorpus and corpus presents a different appearance from that of the isthmus and terminal bulb, there being an oblique line of demarcation between the two seen at the base of the corpus (Fig. 7).

Well developed cardia between the oesophagus and the intestine. Lumen of intestine wide and its walls filled with globules of granular nature. Rectum long and narrow. Rectal glands present (Fig. 7).

Nerve ring surrounds isthmus almost midway between the base of corpus and beginning of terminal bulb, but in some specimens it lies further forward almost at the base of the corpus. Amphids open through an elliptical aperture situated behind main chamber of stoma (Figs. 1, 7). Openings of phasmids situated on slightly raised papilla-like structures symmetrically located on lateral sides of body (Figs. 5, 7).

Excretory pore almost at level of nerve ring or slightly posterior to it, leading into a thin narrow duct directed posteriorly (Fig. 7). A transparent mass of tissue observed surrounding excretory duct.

Two ovaries present, opposed and reflexed. Tips of ovaries cross each other. Oocytes arranged in single file. The two uteri run parallel to reflexed ovaries before joining to open into a short and narrow vagina, the walls of which are lined by refractive cuticle continuous with body cuticle. Vagina opens through a circular vulva placed on top of an elevated vulvar cone. The vulvar cone may be only slightly elevated above the surface of body or the elevation may be quite pronounced. The vulvar aperture, however, appears to be circular in all cases (Figs. 8*a*–8*d*), almost equatorial or slightly post-equatorial in position. Tail narrows gradually to a dorsally hooked terminus (Figs. 5, 7).

Males

Length 1.04–1.37; a=21–35; b=4.3–5.9; c=10.4–11; spicules 0.045–0.052 mm.; gubernaculum 0.017–0.02 mm.

Body assumes shape of a 'J' when killed by gentle heat. Cuticle finely striated, striations being 0.0014 mm. apart at the middle of body.

Head not marked off from rest of body and bears six cirri and six papillae as in female. Stomatorhabdion arrangement similar to that of female.

Cardia between the oesophagus and the intestine. Nature of lumen of oesophagus, the muscular tissue surrounding stoma and oesophagus, and the valve within terminal bulb is similar to that described in female. Intestine narrows considerably as it runs parallel to vas deferens. Cloaca long and narrow lined with thick cuticle. Rectal glands present (Fig. 2).

Single testis extends anteriorly beyond middle of body where it is reflexed. Vas deferens wide and full of sperms (Fig. 2). It narrows posteriorly to open into the cloaca through a short and narrow ejaculatory duct. Two spicules present, of similar shape, and not joined. Gubernaculum present. Tail curved and ending in dorsally hooked terminus. Posterior region of body bears seven pairs of preanal and eight pairs of postanal papillae. Of the latter, two pairs are lateral, five pairs subventral, and one pair subdorsal in position. All preanal pairs subventral in position. No caudal alae.

Diagnosis

Chambersiella with dimensions and general features as described above. Easily distinguished from *C. rodens* by the presence of two ovaries and comparatively smaller buccal hooks.

Host: Bark of oak tree.

Locality: Bank of the Jock River, near Richmond, Ontario.

Type specimens are deposited in the Canadian National Collection of Nematodes, Nematology Section, Science Service Laboratory, Department of Agriculture, Ottawa.

Discussion

The genus *Chambersiella* was established by Cobb in 1920 with *C. rodens* as the type and only species. This single species provided the diagnostic characters of the genus and, later, of the subfamily Chambersiellinae of Thorne, 1937 (5). Obviously Cobb (4) had observed other species as he says, "this genus comprises a considerable number of species, all with a hooked caudal extremity". The mandibles described and figured by Cobb in *C. rodens* are rather large structures with their bases completely covering the head. Cobb says of his species that "it has the strongest mandibles so far known in the genus". In *C. bakeri*, however, the parts called the buccal hooks are relatively smaller structures, clearly contributing to the formation of the anterior part of the stomatal wall, as well as occupying only two-thirds of the head diameter. Unlike the large mandibles arching into a dome-shaped structure in *C. rodens*, the comparatively small buccal hooks of the present form protrude but slightly beyond the head. Another striking difference between *C. bakeri* and *C. rodens* is the presence of two opposed and reflexed ovaries in the former in contrast to the single reflexed ovary in the latter. Further, *C. bakeri* differs from *C. rodens* in the size of the body (attaining almost double the length of the latter) and the position of the vulva.

The genus *Chambersiella* and the subfamily Chambersiellinae have been previously placed in the family Cephalobidae Chitwood and Chitwood, 1934 (1) on the basis of the characters of the oesophagus, the jointed stomatorhabdions which are supposed to be 'apparently panagrolaimoid', the presence of one gonad in the female, and special distribution of caudal papillae in the male. All the genera of Cephalobidae have either a 'panagrolaimoid' or 'cephaloboid' type of stomatorhabdion arrangement and the females have only one ovary. In the absence of a detailed morphological description of the stomatorhabdions in *C. rodens*, it is probably not very safe to conclude on the basis of the figures alone that the stomatorhabdions belong to the 'panagrolaimoid' type. *C. bakeri* does not possess either of the already noted two types of stomatorhabdion arrangement of the Cephalobidae and also it has two ovaries in the female. In addition, *Chambersiella* differs from the genera of the Cephalobidae in the possession of six oral cirri, six 'odontia', in the position of the amphidial apertures (characters that necessitated the creation of a separate subfamily Chambersiellinae). To leave the genus *Chambersiella*

now in the family Cephalobidae would necessitate accepting considerable extensions of the characters of this family and this does not seem to be a satisfactory solution. Disrupting the characters of a well defined family does not seem to be presently justified. When more species of the genus are found and described, its taxonomic position will probably be clearer and any required adjustment can then be made. At the same time the positions of the genera *Alloinema* Schneider, 1859, *Cheilobus* Cobb, 1924, and *Rhabditophanes* Fuchs, 1930 will possibly have to be reconsidered.

Acknowledgments

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LIST OF HELMINTHS FROM ALBERTA RODENTS[1]

GEORGE LUBINSKY

Abstract

Thirty species of helminths were found in material from Alberta rodents and lagomorphs. It was shown that *Capillaria hepatica*, a potential human parasite of high pathogenicity, occurs in *Peromyscus maniculatus* (a new host record) and in *Thomomys talpoides*, and is common throughout the entire province of Alberta.

This list of rodent helminths is the result of identification of material collected by Prof. T. E. Moore of the Department of Zoology of the University of Alberta and sent to Prof. T. W. M. Cameron. The material was collected during the months of May to October of 1952–1955. Helminths, noticed in the course of dissecting the rodents, were preserved in formalin or in formalin – alcohol acetic. The material was collected in 54 localities scattered throughout the entire province from its southern to its northern boundaries. Localities situated between the latitudes of Edmonton and Calgary we have designated as middle Alberta, those situated to the north and south of this belt correspondingly as northern and southern Alberta.

The material contained the following species of helminths:

1. *Andrya macrocephala* Douthitt, 1915
   Microtus pennsylvanicus.—Small intestine; nine localities scattered from northern to southern boundaries of Alberta.
   Pedomys ochrogaster.—Small intestine; middle Alberta.
   Clethrionomys gapperi.—Small intestine; northern and southern Alberta.

2. *Andrya communis* Douthitt, 1915
   Microtus pennsylvanicus.—Small intestine; northern Alberta.
   Clethrionomys gapperi.—Small intestine; southern Alberta and Banff National Park.
   Phenacomys intermedius.—Small intestine; Banff National Park.

3. *Andrya neotomae* Voge, 1946
   Neotoma cinerea.—Small intestine; southeast Alberta.

4. *Andrya arctica* Rausch, 1952
   Synaptomys borealis.—Small intestine; northern Alberta.

5. *Paranoplocephala variabilis* (Douthitt, 1915)
   Thomomys talpoides.—Colon; middle and southern Alberta (six localities).
   Phenacomys intermedius.—Small intestine; Banff National Park.
   Microtus pennsylvanicus.—Small intestine; southern Alberta.

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6. *Paranoplocephala infrequens* (Douthitt, 1915)
Microtus pennsylvanicus.—Northern and middle Alberta.

7. *Schizorchis ochotonae* Hansen, 1948
Ochotona princeps.—Small intestine; Banff National Park.

8. *Catenotaenia dendritica* (Goeze, 1782)
Clethrionomys gapperi.—Small intestine; southern Alberta.

9. *Taenia mustelae* Gmelin, 1790 (larvae)
Microtus pennsylvanicus.—Liver; from northern to southern boundaries of Alberta.
Pedomys ochrogaster.—Liver; southern Alberta.
Clethrionomys gapperi.—Liver; northern to southern Alberta (four localities).
Thomomys talpoides.—Liver, lungs, kidneys, and mesenteries; northern and middle Alberta.

10. *Taenia pisiformis* Bloch, 1780 (larvae)
Lepus americanus.—Mesenteries; southern Alberta.

11. *Hydatigera taeniaeformis* (Batsch, 1786)
Mus musculus.—Liver; southern Alberta.

12. *Cladotaenia mirsoevi* Skrjabin and Popoff, 1924
Clethrionomys sp.—Ileum; middle Alberta.

13. *Hymenolepis horrida* v. Linstow, 1901
Microtus pennsylvanicus.—Small intestine; northern Alberta.
Clethrionomys gapperi.—Small intestine; northern to southern Alberta (four localities).

14. *Hymenolepis diminuta* (Rudolphi, 1819)
Citellus richardsoni.—Small intestine; middle Alberta.

15. *Hymenolepis evaginata* Barker and Andrews, 1915
Ondatra zibethicus.—Intestine; middle Alberta.

16. *Choanotaenia peromysci* (Ericksen, 1938)
Peromyscus maniculatus.—Small intestine; 12 localities from northern to southern boundaries of Alberta.

17. *Choanotaenia nebraskensis* Hansen, 1950
Mus musculus.—Small intestine; northern Alberta.

18. *Choanotaenia* sp.
Zapus hudsonius.—Small intestine; middle Alberta.

19. *Capillaria hepatica* (Bancroft, 1896)
Peromyscus maniculatus.—Liver; 10 localities ranging from the northern to the southern boundaries of Alberta.
Thomomys talpoides.—Liver; southern, middle, and northern Alberta.

20. *Trichuris fossor* Hall, 1916
Thomomys talpoides.—Caecum; middle Alberta.

21. *Syphacia eutamii* Tiner, 1948
Eutamias amoenus.—Large intestine; southern Alberta.

22. *Oxynema* sp.
Citellus tridecemlineatus.—Small intestine; middle Alberta.

23. *Obeliscoides cuniculi* Graybill, 1924
Lepus americanus.—Stomach; middle Alberta.

24. *Heligmosomoides polygyrus* Hall, 1916
Phenacomys intermedius.—Small intestine; Banff National Park.

25. *Protospirura ascaroidea* Hall, 1916
Thomomys talpoides.—Stomach; middle Alberta.

26. *Protospirura muris* Hall, 1916
Clethrionomys gapperi.—Stomach; southern Alberta.

27. *Rictularia coloradensis* Hall, 1916
Microtus pennsylvanicus.—Stomach; northern Alberta.

28. *Physaloptera spinicauda* MacLeod, 1933
Citellus tridecemlineatus.—Stomach; middle Alberta.

29. *Physaloptera bispiculata* Vaz and Pereira, 1955
Ochotoma princeps.—Stomach; southern Alberta.

30. *Litomosoides carinii* (Travassos, 1919)
Thomomys talpoides.—Coelom; middle and southern Alberta.

31. *Haplosporangium parvum* Emmons and Ashburn, 1942
Peromyscus maniculatus.—Lungs; southern Alberta

32. *Sarcosporidia* (unidentified)
Ochotona princeps.—"Muscles of axilla"; southern Alberta.

The distribution of the above parasites among their hosts was as follows:

Rodentia

SCIURIDAE

Citellus tridecemlineatus
Oxynema sp.
Physaloptera spinicauda MacLeod, 1933

Citellus richardsoni
Hymenolepis diminuta (Rud., 1819)

Eutamias amoenus
Syphacia eutamii Tiner, 1948

GEOMYIDAE

Thomomys talpoides
Paranoplocephala variabilis (Douthitt, 1915)
Taenia mustelae Gmelin, 1790 (larvae)
Capillaria hepatica (Bancroft, 1896)
Trichuris fossor Hall, 1916
Protospirura ascaroidea Hall, 1916
Litomosoides carinii (Travassos, 1919)

CRICETIDAE

Cricetinae

Peromyscus maniculatus
Choanotaenia peromysci (Ericksen, 1938)
Capillaria hepatica (Bancroft, 1896)

Neotoma cinerea
Andrya neotomae Voge, 1946

Microtinae

Microtus pennsylvanicus
Andrya macrocephala Douthitt, 1915
A. communis Douthitt, 1915
Paranoplocephala variabilis (Douthitt, 1915)
P. infrequens (Douthitt, 1915)
Taenia mustelae Gmelin, 1790 (larvae)
Hymenolepis horrida v. Linstow, 1901
Rictularia coloradensis Hall, 1916

Pedomys ochrogaster
Andrya macrocephala Douthitt, 1915
Taenia mustelae Gmelin, 1790 (larvae)

Clethrionomys gapperi
Andrya macrocephala Douthitt, 1915
A. communis Douthitt, 1915
Catenotaenia dendritica (Goeze, 1782)
Taenia mustelae Gmelin, 1790 (larvae)
Cladotaenia mirsoevi Skrjabin and Popoff, 1924
Hymenolepis horrida v. Linstow, 1901

Phenacomys intermedius
Andrya communis Douthitt, 1915
Paranoplocephala variabilis (Douthitt, 1915)
Heligmosomoides polygyrus Hall, 1916

Synaptomys borealis
Andrya arctica Rausch, 1952

Ondatra zibethicus
Hymenolepis evaginata Barker and Andrews, 1915

MURIDAE

Mus musculus
Hydatigera taeniaeformis (Batsch, 1786) (larvae)
Choanotaenia nebraskensis Hansen, 1950

ZAPODIDAE

Zapus hudsonius
Choanotaenia sp.

Lagomorpha

Lepus americanus
Taenia pisiformis Block, 1780 (larvae)
Obeliscoides cuniculi Graybill, 1924

Ochotona princeps
Schizorchis ochotonae Hansen, 1948

CHANGES IN RESISTANCE TO DDT IN MACROCENTRUS ANCYLIVORUS ROHW. (HYMENOPTERA: BRACONIDAE)[1]

J. G. ROBERTSON[2]

Abstract

Exposure of the arrhenotokous parasite *Macrocentrus ancylivorus* Rohw. to 23 μg./cm.² of DDT for 3 minutes in each of 10 successive generations increased the level of resistance to four times that of the original stock. When the concentration of DDT was increased to 96 μg./cm.² of DDT for 3 minutes from the F_{11} generation on, the resistance reached a maximum of 12 times that of the initial stock at the F_{19} generation, and at the F_{29} generation was nine times the original level. In the F_{30} to F_{71} generations, when only females were exposed to DDT, the level of resistance fell to seven times its initial value. When, beginning with the F_{72} generation, the parasite was reared for 13 generations without exposure to DDT, resistance fell to its initial level. Females were more DDT-resistant than males.

Introduction

Efforts to select a DDT-resistant strain of the arrhenotokous parasite *Macrocentrus ancylivorus* Rohw. by exposure to a known concentration of *p-p'*-DDT have been reported (16–18). This paper describes the rise and fall of resistance when the concentration of DDT was increased, and the loss of resistance when the parasite was reared without exposure. The entire project covered a period of 6 years and involved more than three million adults.

Methods

Details concerning the rearing, exposure to DDT, and selection for resistance in *M. ancylivorus* have been reported (17–18). About 25–50% of the emerging stock from a daily total of about 1500–3000 adults was exposed to a known concentration of pure *p-p'*-DDT. Selection was begun at a concentration (23 μg./cm.²/3 minutes) which gave an initial survival level of 30%.* The concentration was later increased to 96 μg./cm.² for 3 minutes.

The number of filial generations was estimated on the basis of a 21-day life cycle for *M. ancylivorus* at the rearing temperature of 80° F. Because of the daily breeding operation, each generation was not treated separately, but as an average of 21 stocks.

Beginning with the F_{72} generation, the parasite was reared for 13 generations without exposure to DDT, and tests were then made to determine the possibility of reversion, by the same methods used to determine the initial tolerance (17). The sexes were exposed daily in lots of 50 to a DDT concentration of 23 μg./cm.² for 2, 3, 4, and 6 minutes and to different concentrations of

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*All percentage survival figures adjusted by Abbott's formula except for Fig. 1.

DDT (23, 46, 69 μg./cm.²) for 2 minutes. Controls were run with blank slides. The dead and living were counted at 8 and 16 hours, and at 24-hour intervals thereafter for 10 days. The procedure was repeated each day until 1000 insects had been exposed to each of the conditions. A probit regression analysis and the fiducial limits (6) for the numbers surviving at 40 hours were determined for the series exposed for different times. It is within 40 hours that death due to DDT occurs in *M. ancylivorus* (17).

As data were not available to calculate the mean tolerances (LD_{50}'s) through generations F_{11}–F_{71}, when the DDT concentration was 96 μg./cm.²/3 minutes, they were approximated arithmetically from the known LD_{50} and percentage survival to the same concentration of DDT at the F_{10} generation.

Results and Discussion

The resistance of adults of *M. ancylivorus* to DDT in these selection experiments contrasted sharply with the natural resistance to large concentrations of DDT in the Mexican bean beetle, *Epilachna varivestis* Muls.; the differential grasshopper, *Melanoplus differentialis* Thom.; and the red-banded leaf roller, *Argyrotaenia velutinana* (Wlk.) (7). At the concentration of 96 μg./cm.², peak resistance occurred at the F_{19} generation (Fig. 1, Table I). Here the LD_{50} of 28.2 minutes represented three times the resistance which developed when the concentration was 23 μg./cm.², and approximately 12 times that of the initial stock. This result is similar to the 5- to 10-fold increase in resistance in 6–20 generations of various strains of the housefly, *Musca domestica* L. (4), but not with the 10,000 times increase observed in the *Ax* strain at the F_{20} generation (19). However, it was higher than the two- to three-fold increase reported for the Syosset lines of *Drosophila melanogaster* Meig. after 20–22 generations of selection (9, Table 1). These similarities

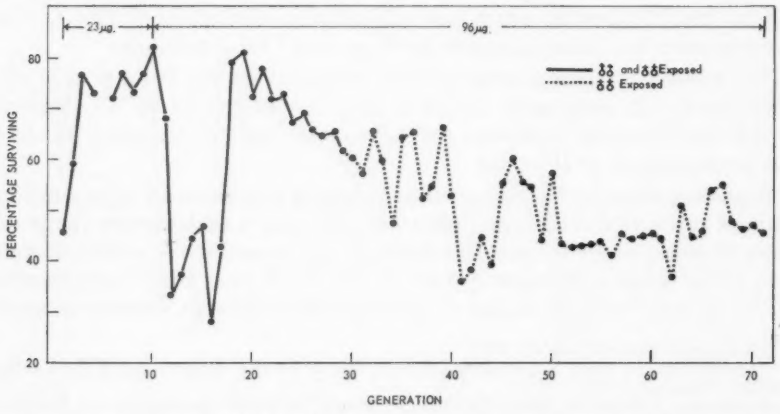

FIG. 1. Selection for DDT resistance in *Macrocentrus ancylivorus* Rohw. exposed to two concentrations of DDT (in μg./cm.²/3 minutes). No exposure in the F_5 generation.

TABLE I

MEAN TOLERANCES (LD_{50}'S IN MINUTES) CALCULATED AT 40 HOURS FOR *Macrocentrus ancylivorus* ROHW. EXPOSED TO DDT CONCENTRATIONS OF 23 μG./CM.² (PARENT; F_1 TO F_{10} AND F_{85} GENERATIONS) AND 96 μG./CM.² (F_{11} TO F_{71} GENERATIONS)*

		Generation				
	Parent	F_{10}	F_{19}	F_{29}	F_{41-71}	F_{85}
Male	2.0	7.5	—	—	—	2.5 (2.4–2.7)†
Female	2.6	11.3			16.9	3.0 (2.9–3.2)
Mean	2.3	9.4	28.2	20.7	—	2.7

*Both sexes exposed from F_1 to F_{29} generations; only females from F_{30} to F_{71}; neither from F_{72} to F_{84}. Parent and F_{10} values from Pielou and Glasser (18).
†95% fiducial range.

or differences in response to DDT may be fortuitous as the methods of exposure (2) and the concentrations of the insecticides to which the insects are exposed (10) affect the results (4).

The resistance in *M. ancylivorus* gradually declined from the peak obtained at the F_{19} generation to nine times the initial level at the F_{29} generation. This decline in resistance corrects the tentative observation (21) that resistance was increasing in this period. Comparable observations are infrequent, but King (8) noted that resistance of the SYS-1 line of *D. melanogaster* fell to its initial level despite continued selection. These results suggest that resistance may be associated with factors harmful to survival. In houseflies, Maelzer and Kirk (13) found that low fertility was associated with the high resistance factor in the Multi-*x* strain. This association was absent in the DDT-1 strain (12).

In the 42 generations (F_{30}–F_{71}) of *M. ancylivorus* in which only females were exposed to DDT, survival fluctuated considerably (Fig. 1) and resistance declined to about seven times that of the original stock (Table I).

When the parasites were reared for 13 generations (F_{72}–F_{84}) without exposure to DDT, the resistance reverted to practically the initial level (Table I). In houseflies reared without exposure to DDT, the DDT-resistant Orlando strain showed a decline in resistance when tested at the F_9 and F_{14} generations (11) and the highly resistant *Ax* strain lost its resistance in 22 generations (19). The Bellflower and Pollard DDT-resistant strains maintained resistance for 15–20 generations free from exposure to DDT (14), and the DDT-1 strain was resistant after 34 generations (1). In *D. melanogaster* no appreciable loss of resistance occurred when exposure to DDT was stopped for 3 years (2), and the California red scale, *Aonidiella aurantii* (Mask.), showed no loss in resistance to nitrogen cyanide after 150 unexposed generations (15, p. 206, unpublished information of Lindgren, D.L.). Apparent differences in these findings probably reflect the degree of genetic stability established prior to tests for reversion.

Females of *M. ancylivorus* were more resistant than the males (Table I). It was not determined whether this was a consequence of the haplodiploidy in this species.

A study of the life cycle of *M. ancylivorus* (5) strongly suggests that the germ cells were not exposed to DDT action before maturation, though cytological evidence is lacking. Resistance in this species, therefore, appears to support the preadaptation theory (3, 15). Though most work on the inheritance of resistance shows that resistance is polygenic, there is no evidence concerning the nature of the genetic mechanism of *M. ancylivorus*. In another arrhenotokous species, the two-spotted spider mite, *Tetranychus telarius* (L.), Taylor and Smith (20) showed that resistance is controlled by a single dominant factor.

Acknowledgments

Dr. D. P. Pielou, Entomology Laboratory, Summerland, British Columbia, initiated the project and published on his work until leaving the Entomology Laboratory, Belleville, Ontario. I am greatly indebted to Dr. Pielou for permission to use his unpublished data with those I obtained. Valuable assistance was rendered by Mr. J. A. Calvert of the Belleville laboratory throughout the program. Misses R. Webb and E. Bawden helped in the rearing, exposure, and counting aspects of the work.

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